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Permeation of WIN 55,212-2, a potent cannabinoid receptor agonist, across human tracheo-bronchial tissue in vitro and rat nasal epithelium in vivo

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Abstract

The aim of this study was to investigate the intranasal absorption of *R*-(+)-WIN 55,212-2 mesylate in vivo and in vitro. Permeation experiments of *R*-(+)-WIN 55,212-2 formulations with 2% dimethyl-β-cyclodextrin (DMβCD), 2% trimethyl-β-cyclodextrin (TMβCD) or 2% randomly methylated-β-cyclodextrin (RAMβCD) in 1:1 propylene glycol/saline and 1.5% propylene glycol + 3% Tween 80 in saline were conducted using EpiAirway™ tissue and an anesthetized rat nasal absorption model, respectively. Samples were analysed by liquid chromatography-mass spectrometry. Mucosal tolerance was screened using paracellular marker permeation and tissue viability as indices. Nasal absorption of WIN 55,212-2 was rapid, with a t_{max} (time of peak concentration) of 0.17 to 0.35 h in vivo. Relative to 1.5% propylene glycol + 3% Tween 80 (control), 1:1 propylene glycol/saline, RAMβCD, DMβCD and TMβCD resulted in 24-, 20-, 17- and 10-fold WIN 55,212-2 permeation increases in vitro, respectively. The in vivo absolute bioavailabilities were also increased with 1:1 propylene glycol/saline, RAMβCD, DMβCD and TMβCD compared to 1.5% propylene glycol + 3% Tween 80 (0.15 vs. 0.66–0.77). The viability of the EpiAirway™ tissues was significantly reduced by DMβCD and TMβCD formulations. This study showed that WIN 55,212-2 mesylate can be delivered via the nasal route. Absorption of *R*-(+)-WIN 55,212-2 was rapid and bioavailability was significantly improved using methylated cyclodextrins and propylene glycol-based cosolvent.

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Introduction

The pharmacological actions of cannabinoids are mainly due to the interaction of the drugs with cannabinoid receptors located in the central and peripheral nervous systems. The therapeutic benefits arising from such interaction include analgesia, appetite stimulation, muscle relaxation, intraocular pressure reduction and antineoplastic, anti-emetic, anti-inflammatory and neuroprotective effects (Adams & Martin 1996).

R-(+)-WIN 55,212-2 ((4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H* pyrrolo [3,2,1ij] quinolin-6-one; Figure 1) is a non-classical synthetic cannabinoid (devoid of phytocannabinoid ring structures). This potent cannabinoid prevents intravenous cocaine self-administration, increases tail-flick reflexes, exerts antihyperalgesic and anti-inflammatory effects, and induces hypothermia in rats (Showalter et al 1996; Rawls et al 2002; Pozzi et al 2003). Co-administration of WIN 55,212-2 and morphine produced greater antihyperalgesia at a lower dose than either drug administered alone (Nguyen et al 2002). This strategy could be advantageous in certain types of cancer, especially since WIN 55,212-2 also has the potential to inhibit tumour growth (Casanova et al 2003). For acute cancer pain management, however, fast onset of drug action is desirable. Considering the rapid onset of drug action following nasal administration (Hussain et al 1980; Coda et al 2003), which sometimes superimposes intravenous (i.v.) doses, the nasal route appears to be suitable for systemic administration of cannabinoids. For therapeutic purposes, cannabinoids (mostly tetrahydrocannabinol) have been investigated for transdermal, rectal, sublingual, oral, ophthalmic and inhalation delivery (Grotenhermen 2003). There are no publications in the literature about the systemic delivery of WIN 55,212-2 via the nasal route.

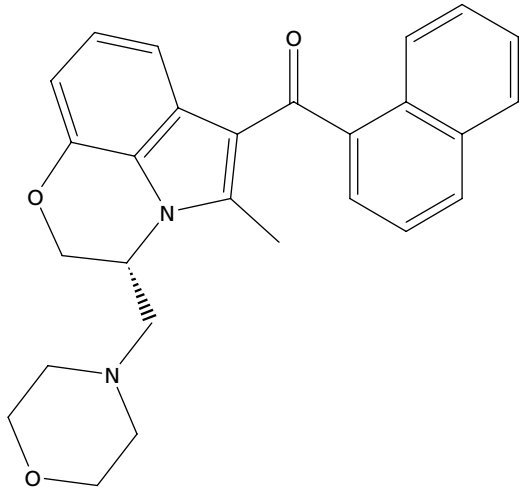

Figure 1 Chemical structure of WIN 55,212-2 ($C_{27}H_{26}N_2O_3$; mol. wt 426.51; clogP 4.28 ± 1.3; pKa 7.1 ± 0.2). Calculated logP from Advanced Chemistry Development Software Solaris V4.76.

WIN 55,212-2 is a very hydrophobic drug and therefore requires some solubilization formulation strategies before it can be delivered intranasally. Several options, including the use of suspensions, solubilizing agents, co-solvents and cyclodextrins, have been used to formulate insoluble drugs for nasal administration (Merkus et al 1999; Li et al 2000; Hochhaus et al 2002). In this study, propylene glycol-based co-solvents and methylated cyclodextrins were used to prepare WIN 55,212-2 formulations. For in vivo studies, an anesthetized rat model (Hussain at al 2000) was used. Based on earlier studies (El-Shafyl et al 2001; Quay et al 2001; Scotto-Lavino et al 2002), the EpiAirway™ tissue model was chosen for in vitro evaluation of the nasal absorption potential of WIN 55,212-2 formulations, as well as to determine the possible detrimental effects that may be caused by the drug and/or excipients used in the formulations. The EpiAirway™ system consists of human-derived tracheal/bronchial epithelial cells grown on a collagen-coated membrane to form a well-differentiated, organotypic model with many of the same features of nasal mucosa.

Materials and Methods

Chemicals

Sodium fluorescein was purchased from Aldon Corporation (Avon, NY), WIN 55,212-2 mesylate was purchased from Sigma (St Louis, MO), dimethyl-β-cyclodextrin (DMβCD) was purchased from Acros (Morris Plains, NJ) and randomly methylated cyclodextrin (RAMβCD) and trimethyl-β-cyclodextrin (TMβCD) were purchased from CTD Inc. (High Springs, FL). Dulbecco's phosphate buffered saline (PBS, without calcium chloride and magnesium), Air-100-MM maintenance medium, MTT concentrate (MTT-100-CON 2 mL (5 mg mL^{-1})) and MTT diluent/extractant solution (MTT-100-EXT) were generous gifts from MatTek Corp. (Ashland, MA). Propylene glycol, ethanol and Tween 80 were obtained from Fischer Scientific Company (Fairlawn, NY), Aldrich Chemical Company (Milwaukee, WI) and ICN Biomedical (Aurora, OH), respectively.

Preparation of formulations for in vivo and in vitro experiments

Preparation of i.v. formulation

A weighed amount of WIN 55,212-2 was dissolved in propylene glycol (5% of the total volume) and the solution was vortexed and sonicated for 5 min. The volume was made up with sterile saline containing 3% v/v Tween 80 to give a 450 μg mL^{-1} solution. The solution was vortexed and sonicated for 5 min. Drug solutions were prepared immediately before each animal was dosed.

Preparation of intranasal (i.n.) formulations

A weighed amount of WIN 55,212-2 (for a final concentration of 4.5 mg mL^{-1}) was dissolved in a 1:1 v/v propylene glycol/saline solvent system, or a 1:1 v/v propylene glycol/saline solvent system with 2% w/v cyclodextrin, or 1.5% propylene glycol and saline containing 3% Tween. Solutions were vortexed and sonicated for 5 min.

Preparation of formulations for in vitro studies

A weighed amount of the drug was dissolved in propylene glycol (1.5% of the total volume or 50% of the total volume). The solutions were vortexed and sonicated for 5 min. The volume was made up with sterile saline or sterile saline containing 3% v/v Tween 80 or cyclodextrins (2% w/v of total volume) to give a final concentration of 200 μM.

Rat surgery, drug administration and sample collection

The animal studies were approved by the University of Kentucky Institutional Animal Care and Use Committee. All animal procedures were conducted according to *Principles of laboratory animal care* (NIH publication no. 85-23). The animals (three or more rats per group) were prepared for pharmacokinetic studies using a surgical procedure allowing intranasal administration as described by Hussain et al (2000). Briefly, male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 240–345 g were anesthetized with intraperitoneally administered ketamine (100 mg kg^{-1}) and xylazine (5 mg kg^{-1}). A vertical midline incision of approximately 2 cm was made in the throat to isolate the trachea and esophagus. The trachea was cannulated and the esophagus blocked towards the posterior part of the nasal cavity to prevent nasal drainage into the stomach. The nasopalatine passage was closed with cyanoacrylate glue, which prevents nasal drainage into the mouth. Furthermore, the right jugular vein and left femoral vein (when dosing i.v. formulations) were exposed and cannulated with medical grade silastic tubing (0.020″ i.d. × 0.037″ o.d.). Each rat was administered WIN 55,212-2 formulations (150 μg kg^{-1}) intranasally using a 25-μL gas-tight Hamilton syringe via PE-50 tubing. For intravenous administration, the dose was

delivered into the cannulated femoral vein via the catheter over a period of 30 s. After dosing, the femoral line was flushed with vehicle and sterile saline (0.2 mL). Blood samples (0.3 mL) were drawn from the jugular vein at regular time intervals up to 240 min for i.v. and i.n. doses. The experiment was carried out for 4 h because our preliminary experiments showed that WIN 55,212-2 was not detectable beyond this time point. The samples were transferred to 1.5-mL siliconized microcentrifuge tubes containing 5 μL of heparin (1000 U mL^{-1}). The samples were immediately centrifuged (4000 rpm × 15 min) and the collected plasma samples were stored at −70°C.

Tissue culture

The EpiAirway™ tissues (Air-100) used for the studies were supplied by MatTek Corp. (Ashland, MA). On delivery, the tissues were processed according to the supplier's protocol. Briefly, each tissue was aseptically transferred to a well in six-well plates already filled with 2 mL of Air-100-MM maintenance medium. The tissues were maintained at the air–liquid interface and were allowed to equilibrate at 37°C in the presence of 5% CO_2 for 24 h prior to the permeation studies.

Permeation studies

Immediately before the permeation studies, the tissues were rinsed twice with Dulbecco's PBS (without calcium and magnesium) and were pre-incubated with Hanks' balanced salt solution supplemented with 10 mM HEPES buffer and 25 mM glucose (pH 7.4) for 30 min at 37°C. Transport studies were initiated by adding 0.5 mL of the test solution to the donor compartment and 2.0 mL of the supplemented Hanks' balanced salt solution containing 4% bovine serum albumin (BSA, transport medium) to the receiver side. At predetermined time points (0–120 min), 100 μL aliquots were sampled from the receiver compartment and replaced immediately with an equal volume of transport medium. The receiver solution was continuously mixed with the aid of micro-magnetic stirrers (100 rpm). All tissues used were checked for epithelial integrity before and after the experiments by measuring transepithelial electrical resistance (TEER) and sodium fluorescein (1 mg mL^{-1} for 1 h) transport at the end of the experiments as well. The electrical resistance of the tissues was measured using an EVOM Epithelial Voltohmmeter, which was connected to an endohm chamber (World Precision Instruments, Sarasota, FL). The TEER was corrected for background values due to the filter membrane and collagen coating. Apparent permeability coefficients (P_{app} (cm s^{-1})) for the formulations were calculated using a standard equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{V}{AC_0}$$

where dQ/dt (μg s^{-1}) is the steady rate of appearance of the test compound to the basolateral side, C_0 (μg mL^{-1}) is the initial concentration in the apical chamber (200 μM), A (cm^2) is the effective tissue surface area of the inserts (1 cm^2) and V (mL) is the volume of the receiver compartment (2.0 mL).

Sample extraction and analysis

Exactly 500 μL of acetonitrile was added to a 50-μL aliquot of sample in a 1.5-mL siliconized microcentrifuge tube, and the mixture was vortexed for 30 s and centrifuged at 10 000 × g for 20 min. The supernatant was decanted to 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 onto the column of the liquid chromatography-mass spectrometry (LC–MS) system.

LC–MS conditions

The liquid chromatograph was a Waters Alliance 2690 HPLC pump (Waters, Milford, MA) with a Waters Alliance 2690 autosampler and a thermostatic column compartment. The analytical column was a Waters Symmetry C_{18} (2.1 × 150 mm, 5 μm) and the guard column was a Waters Symmetry C_{18} (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^{-1}. 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, Milford, MA) equipped with an electrospray ionization probe. Selected ion monitoring 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 and 250°C, respectively. Nitrogen was used as a nebulizator and drying gas at flow rates of 50 and 450 L h^{-1}, 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 100–4000 ng mL^{-1}, and the observed correlation coefficient was consistently ≥ 0.999.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolinium bromide (MTT) assay

The MTT assay was used to monitor the viability of the tissues following exposure to the test formulations. The assay was conducted using MTT-100 MatTek kits according to the company's protocol. Briefly, at the end of each permeation study, each insert containing the tissue was transferred to a well containing 0.6 mL of the MTT solution. The tissues were incubated for 2 h at 37°C. Mitochondrial dehydrogenase (MDH) enzyme activity, as reflected by formazan formation, was used to assess the viability of the tissues. Formazan crystals were extracted from each tissue using 4 mL of MatTek's extractant solution in overnight incubation. Formazan optical density was measured spectrophotometrically at 570 nm using the extractant solution as the blank solvent. To improve the quality of the data, an optical density adjustment was made by subtracting optical density values obtained at 650 nm from the values at 570 nm.

Data treatment

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). Data from intranasal administration were fitted to a one-compartment model with absorption phase. The pharmacokinetic parameters, such as absorption half-life, $t_{1/2(ka)}$, distribution half-life, $t_{1/2(\alpha)}$, elimination half-life, $t_{1/2(\beta)}$, steady-state volume of distribution, V_{ss}, area under the curve from 0 to infinity, $AUC_{0-\infty}$, time to reach the maximum concentration, t_{max}, and total body clearance, CL, were estimated using the software.

Statistical analysis of the in vivo data obtained after intravenous or intranasal administration, including the effect of formulations on the pharmacokinetic parameters, was performed with a Kruskal–Wallis test followed by Dunn's test using SigmaStat software. The level of significance was $P<0.05$.

Unless otherwise stated, each in vitro experiment was performed in triplicate using different tissues and the data were expressed as mean ± s.d. The Kruskal–Wallis test followed by Dunn's test was used to estimate the effects of the formulation excipients on sodium fluorescein permeation and tissue viability relative to tissues treated with normal saline. The level of significance was set at $P<0.05$.

Results and Discussion

It has been demonstrated that WIN 55,212-2 and other potent synthetic cannabinoids are good drug candidates for the development of transdermal therapeutic systems for treatment of chronic conditions (Valiveti et al 2004). According to these studies, the transdermal absorption of these compounds exhibits long lag times. In order to use cannabinoids for the treatment of medical conditions requiring immediate onset of drug action (e.g. acute pain management in cancer and other diseases), it is necessary to explore alternative routes of delivery for these compounds. In this study, the intranasal delivery of propylene glycol- and cyclodextrin-based formulations of WIN 55,212-2 was investigated using in vivo and in vitro approaches.

In vivo pharmacokinetic studies

Due to the hydrophobic nature of WIN 55,212-2, methylated cyclodextrins and co-solvents were used to improve the aqueous solubility of the compound and possibly to enhance its nasal absorption as well. Propylene glycol is one of the most widely used cosolvents for improving the aqueous solubility of hydrophobic drugs. Figure 2 shows the time-course of plasma concentrations of WIN 55,212-2 ($150\ \mu g\ kg^{-1}$ dose) after i.n. dosing with formulations containing 1.5% propylene glycol + 3% Tween 80 and propylene glycol/saline (1:1) compared to the intravenous dose profile. The plasma concentration–time data were corrected for animal weight and fitted to a one-compartment pharmacokinetic model with absorption phase using WinNonlin. The nasal absorption of WIN 55,212-2 formulated with 1.5% propylene glycol + 3% Tween 80 was rapid ($\alpha = 0.04 \pm 0.01\ h^{-1}$, $t_{max} = 0.17 \pm 0.01$ h). The

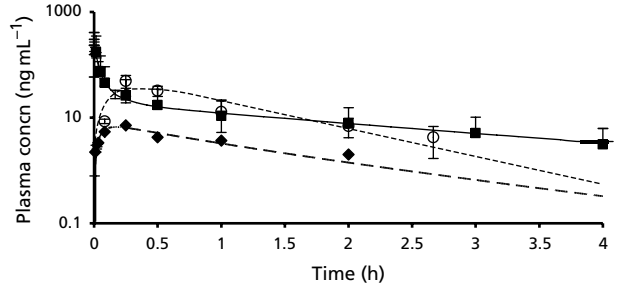

Figure 2 Plasma concentration–time profiles of i.n. (n = 3) and i.v. (n = 6) administration of WIN 55,212-2 formulations without cyclodextrins. ♦, PG + Tween observed; ○, PG/saline observed; ■, i.v. observed; – – – –, PG + Tween predicted; - - - - , PG/saline predicted; ——, i.v. predicted. PG, propylene glycol. Data represent mean ± s.d.

AUC, C_{max} (maximum plasma concentration) and bioavailability for the formulation were $9.01 \pm 1.86\ ng\ mL^{-1} \cdot h$, $6.64 \pm 1.28\ ng\ mL^{-1}$ and 15%, respectively. Increasing the concentration of propylene glycol to 50% resulted in a five-fold increase in AUC, C_{max} and bioavailability, and a larger t_{max} and absorption half-life following nasal administration (Table 1). Although the systemic clearance after nasal administration of the propylene glycol/saline (1:1) formulation ($3.44 \pm 0.89\ L\ h^{-1}$) was comparable to i.v. doses of 1.5% propylene glycol + 3% Tween 80 formulation ($3.38 \pm 2.08\ L\ h^{-1}$), the clearance of WIN 55,212-2 formulated with propylene glycol and Tween 80 following nasal administration was faster ($8.69 \pm 2.3\ L\ h^{-1}$).

Methylated cyclodextrins have been shown to be important absorption enhancers for the nasal delivery of different classes of compounds (Merkus et al 1999). The permeability enhancing effect of cyclodextrins is the result of their interaction with the nasal epithelial membranes and their ability to transiently open tight junctions (Marttin et al 1998). The plasma profiles of WIN 55,212-2 after i.n. dosing with formulations containing propylene glycol/saline (1:1) alone and with cyclodextrins are shown in Figure 3. The AUC of the propylene glycol/saline (1:1) formulation ($45.39 \pm 10.29\ ng\ mL^{-1} \times h$) was comparable to that of WIN 55,22–2 formulations with cyclodextrins ($36.22–39.27\ ng\ mL^{-1} \times h$). The inclusion of 2.0% methylated cyclodextrins did not appreciably alter the absorption half-life, C_{max}, t_{max}, steady-state volume of distribution or extent of absorption ($P > 0.05\%$) (Table 1). This may be because of possible interference with the mechanisms of absorption enhancement of cyclodextrins by the high concentration of propylene glycol in the formulations. The absolute bioavailabilities were 0.77, 0.61, 0.67 and 0.66 for propylene glycol/saline (1:1), RAMβCD, DMβCD and TMβCD, respectively.

In vitro permeation studies with EpiAirway™ tissues

A number of studies where in vivo nasal absorption in various animal models was correlated with human data have been reported (Lindhardt et al 2002; Wadell et al 2003). Because of

Table 1 Summary of pharmacokinetic parameters of WIN 55,212-2 formulations following i.v. and i.n. administrations

Pharmacokinetic parameter	Intravenous[a]	Intranasal[b]				
	1.5% PG + 3% Tween 80*	1.5% PG + 3% Tween 80	PG/saline (1:1)	PG/saline (1:1) + 2% DMβCD	PG/saline (1:1) + 2%RAMβCD	PG/saline (1:1) + 2% TMβCD
AUC (ng mL^{-1} × h)	58.69 ± 30.36	9.01 ± 1.86[1]	45.39 ± 10.29	39.27 ± 2.88	36.22 ± 10.8	38.9 ± 2.40
$t_{1/2}$ (α) (h)	0.08 ± 0.06					
$t_{1/2}$ (β) (h)	3.03 ± 3.22	0.85 ± 0.35	0.57 ± 0.11	0.54 ± 0.38	0.46 ± 0.28	0.78 ± 0.08
$t_{1/2}$ (ka) (h)	–	0.04 ± 0.01	0.13 ± 0.06	0.23 ± 0.23	0.15 ± 0.10	0.09 ± 0.01
C_{max}(ng mL^{-1})	234.3 ± 186.0	6.6 ± 1.3†	35.7 ± 7.2	29.9 ± 5.6	34.9 ± 10.8	26.1 ± 1.5
t_{max} (h)	–	0.17 ± 0.01†	0.35 ± 0.07	0.31 ± 0.37	0.29 ± 0.04	0.32 ± 0.05
CL (L h^{-1})	3.38 ± 2.08	8.69 ± 2.3*	3.44 ± 0.89	3.83 ± 0.28	4.14 ± 0.73	3.86 ± 0.24
V_{ss} (L kg^{-1})	11.08 ± 9.52					
Weight (kg)	320.0 ± 36.4	239.5 ± 11.0	342.8 ± 7.7	295.5 ± 35.3	343.8 ± 8.3	322.7 ± 7.8
F	1.00	0.15 ± 0.03	0.77 ± 0.34	0.67 ± 0.09	0.61 ± 0.05	0.66 ± 0.04

Data represent mean ± s.d. PG, propylene glycol; $t_{1/2}$ (α), distribution half-life; $t_{1/2}$ (β), elimination half-life; $t_{1/2}$ (ka), absorption half-life; F, bioavailability; V_{ss}, apparent volume of distribution at steady state; [a]n = 6, [b]n = 3. *Significantly different compared to i.v. dosing, propylene glycol/saline alone and with cyclodextrins ($P < 0.05$). †Significantly different compared to propylene glycol/saline alone and with cyclodextrins ($P < 0.05$).

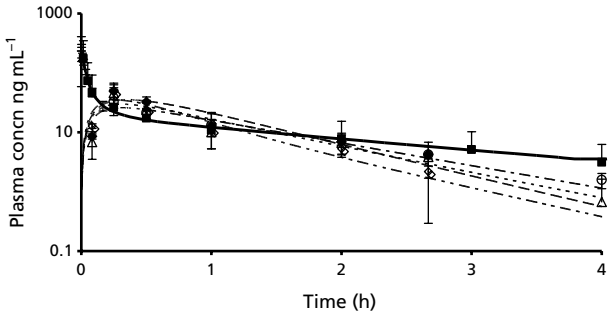

Figure 3 Plasma concentration–time profiles after i.n. (n = 3) and i.v. (n = 6) administration of WIN 55,212-2 formulations with propylene glycol and methylated cyclodextrins. ■, i.v. observed; ♦, PG/saline observed; △, DMβCD observed; ○, TMβCD observed; ◇, RAMβCD observed. PG, propylene glycol. Data represent mean ± s.d.

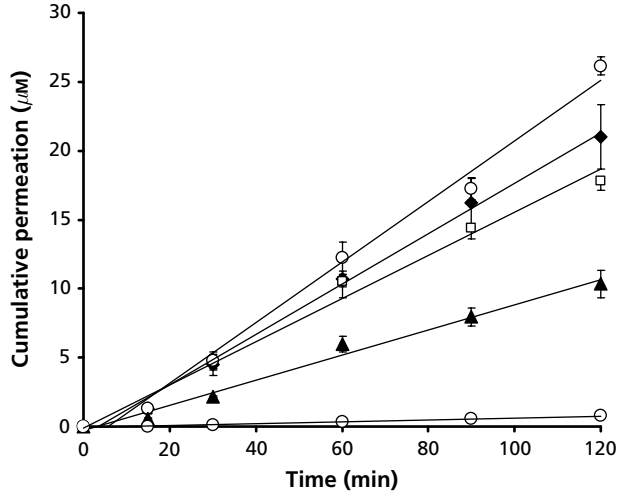

Figure 4 Permeation of WIN 55,212-2 across EpiAirway™ tissues. ♦, WIN 55,212-2 + 2% RAMβCD; □, WIN 55,212-2 + 2% DMβCD; ○, WIN 55,212-2 + PG/saline 1:1; ▲, WIN 55,212-2 + 2% TMβCD; ○, WIN 55,212-2 + 1.5% PG + 3.0% Tween 80. Formulations with cyclodextrins also contain 50% PG. PG, propylene glycol. Data represent mean ± s.d., n = 3. Increasing the propylene glycol concentration to 50% (alone or with cyclodextrins) increased the apparent permeability coefficient by 10–24-fold ($P < 0.05$).

the cost and complexity of in vivo studies, in vitro models involving excised animal tissues or cultured human cells have recently been used to study nasal drug absorption, metabolism and absorption enhancement (Agu et al 2002; Wadell et al 2003). Studies involving human nasal tissues or cultured cells are not commonly used in preclinical drug development studies mainly because human nasal biopsies are difficult to obtain and when the tissues are available, establishing polarized nasal culture systems for drug permeation studies is very difficult. To alleviate these problems, it has been reported that commercially available tracheo-bronchial tissues (EpiAirway™) are suitable for preclinical evaluation of nasal drug formulations (El-Shafyl et al 2001; Quay et al 2001; Scotto-Lavino et al 2002). Besides the fact that the nasal, tracheal and bronchial epithelia are of the same progeny and morphologically similar (Devalia et al 1990; Devalia & Davies 1991), recent studies have demonstrated that the EpiAirway™ cell culture system shows good drug absorption correlation to bovine excised tissue, a popular in vitro nasal model that exhibits absorption and metabolism characteristics similar to that of the human species (Schmidt et al 1998; Chemuturi et al 2003). Figure 4 shows the permeation of different WIN 55,212-2 formulations across the EpiAirway™ tissues. The apparent permeability coefficient for apical to basolateral transport of WIN 55,212-2 formulated with 1.5% propylene glycol + 3.0% Tween 80 was $1.51 \pm 0.2 \times 10^{-6}$ cm s^{-1}. Increasing the propylene glycol concentration to 50.0% resulted in a 24-fold ($P_{app} = 36.6 \pm 8.2 \times 10^{-6}$ cm s^{-1}) increase in WIN 55,212-2

permeation. Co-administration of WIN 55,212-2 in 50% propylene glycol formulation with trimethyl-O-beta, randomly methylated and dimethyl-beta cyclodextrins resulted in a 10- ($P_{app} = 15.4 \pm 1.2 \times 10^{-6}$ cm s^{-1}), 20- ($P_{app} = 30.5 \pm 3.0 \times 10^{-6}$ cm s^{-1}) and 17- ($P_{app} = 26.0 \pm 1.0 \times 10^{-6}$ cm s^{-1}) fold increase in the amount of WIN 55, 212–2 transported across the epithelium.

It can be seen from these results that the permeation of WIN 55,212-2 increased when the formulation content of propylene glycol increased from 1.5 to 50% both in vivo and in vitro, although the extent of enhancement differed greatly and was less significant in vivo. Addition of cyclodextrins to the 50% propylene glycol formulation of WIN 55,212-2 did not result in increased permeation when compared to the 50% propylene glycol alone. The permeation of WIN 55,212-2 from different cyclodextrin formulations varied significantly across tracheo-bronchial tissues, whereas no differences in the rate or extent of absorption from these formulations were found in rats.

Absorption enhancement and toxicity are key issues in designing efficacious and safe medications for the nasal route. In this study the permeation of sodium fluorescein, a paracellular marker, was used to assess the epithelial membrane integrity following exposure to the WIN formulations. The MTT assay was conducted to assess whether possible changes in the membrane integrity of the tissues could be due to the cytotoxic effects of the formulations. The results of these studies are shown in Table 2. The sodium fluorescein permeation values in tissues treated with normal saline, a mixture of 1.5% propylene glycol + 3% Tween 80 and 1:1 propylene glycol/saline without WIN 55,212-2 were 0.3 ± 0.2, 0.4 ± 0.2 and 9.2 ± 0.7% h^{-1}, respectively. WIN 55,212-2 formulations prepared with 50% propylene glycol and cyclodextrins resulted in sodium fluorescein flux in a range of 2 to 18% h^{-1}. Although the sodium fluorescein fluxes, especially for some formulations containing propylene glycol/saline (1:1) and some cyclodextrins, may suggest possible epithelial membrane perturbation, it is important to note that the detrimental effects of the formulations would be attenuated in vivo due to: (i) protection of the nasal epithelium in vivo by mucus secretion, (ii) dilution of administered drug by secreted mucus and (iii) rapid clearance of the administered drug by the mucociliary clearance system. Table 2 also summarizes the effects of WIN 55,212-2 formulations on the mitochondrial dehydrogenase activity of the EpiAirway™ tissues. The formulations containing 2.0% DMβCD and TMβCD significantly reduced the mitochondrial dehydrogenase activity of the EpiAirway™ tissues. No statistically significant difference was observed with respect to the enzyme activity of the formulations prepared with propylene glycol/saline (1:1) alone and the one containing RAMβCD ($P > 0.05$). Considering the fact that incorporation of cyclodextrins in WIN 55,212-2 formulations did not result in improved WIN 55,212-2 absorption, but rather was detrimental in some formulations; one may conclude that WIN can be delivered effectively with propylene glycol alone via the nasal route.

Propylene glycol has been shown to have beneficial effects in the treatment of perennial rhinitis (Spector et al 1982). However, in vivo subchronic studies in rats following nasal inhalation showed that high concentrations of the compound may lead to an increase in the number of goblet cells or an increase in the mucin content of the existing goblet cells in the nasal passages due to irritation and dehydration of the nasal epithelium (Suber et al 1989). However, this may be avoided by introducing hydrophilizing agents into formulations containing high concentrations of propylene glycol, as has been demonstrated for the oral cavity (Kubis & Malecka 1988). Nonetheless, because of the high proportion of propylene glycol used in the formulation and its potential risks in clinical application, other glycols (like polyethylene glycol) might need to be evaluated for an alternative pharmaceutical formulation of the compound. Although up to 2% of methylated cyclodextrins have been safely used for nasal drug delivery (Merkus et al 1999), the detrimental effects seen in this study may be ascribed to an additive or synergistic effect when co-administered with WIN 55,212-2.

It should also be noted that the administration of psychoactive cannabinoids is not without potential side-effects and abuse liability. Recent studies have shown that WIN 55,212-2 significantly increased intravenous self-administration in mice (Martellotta et al 1998) and rats (Fattore et al 2001). Animal self-administration behaviour is a method for

Table 2 Effects of WIN 55,212-2 formulations on sodium fluorescein permeation and mitochondrial dehydrogenase enzyme activity in EpiAirway™ tissues

Formulations	Sodium fluorescein permeation (% h^{-1})	MTT assay	
		optical density$^{570–650}$	MDH activity (% of initial value)
Formulation solvents			
0.9% sodium chloride (control)	0.3 ± 0.2	1.08 ± 0.09	–
Propylene glycol/saline (1:1)	9.2 ± 0.7	0.90 ± 0.09	84.3 ± 8.4
1.5% propylene glycol + 3% Tween 80	0.4 ± 0.2	1.01 ± 0.07	94.7
Drug (200 μM) with excipients			
WIN 55,212-2 + 2.0% DMβCD[a]	2.4 ± 3.2	0.27 ± 0.29	25.3 ± 27.2*
WIN 55,212-2 + 2.0% TMβCD[a]	17.7 ± 0.5	0.18 ± 0.04	16.4 ± 4.1**
WIN 55,212-2 + 2.0% RAMβCD[a]	13.8 ± 6.7	0.88 ± 0.02	82.5 ± 1.8
WIN 55,212-2 in propylene glycol/saline	15.0 ± 2.7	0.89 ± 0.07	81.9 ± 8.1

[a]Formulations also contain 50% propylene glycol. *Significantly different ($P < 0.05$). **for significant difference.

studying the reinforcing properties of drugs and for predicting their abuse potential. The intranasal delivery of this drug may result in rapid absorption and high concentrations in the brain, causing significant pain relief in terminally ill patients, but also a potential dosage form of abuse in non-patients.

Conclusions

In the present study, significant improvement in the nasal absorption of WIN 55,212-2 was achieved using propylene glycol and methylated cyclodextrins when compared to the saline control formulation (PG+TW80). Nevertheless, some of the formulations containing cyclodextrins were found to be detrimental to the epithelium in vitro. By systematic adjustment of propylene glycol and cyclodextrin ratios, nasal formulations that are less deleterious to the nasal mucosa may be designed.

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