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Comparative diffusion of drugs through bronchial tissue

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Abstract

The purpose of the study was to investigate the molecular diffusion of drugs across porcine bronchial tissue. Using an *in vitro* flow-through diffusion system, a series of model compounds were tested. These included theophylline, caffeine, theobromine, enprofylline, salbutamol, ipratropium bromide, and trimethoprim. All drugs were assayed by HPLC in conjunction with UV/vis or MS/MS detection. The results indicated that the mean flux value of theophylline was higher than that of all the other drugs listed above. Within the $\log_{10} P$ range from −2.21 (ipratropium bromide) to 1.364 (trimethoprim), a sigmoidal relationship was found to exist between the apparent permeability coefficients (P_{app}) and the octanol/water partition coefficients across the bronchial tissue. The diffusion of ipratropium bromide (P_{app} 1.6×10^{-8} cm/s) across bronchial tissue was similar to that of salbutamol (P_{app} 1.5×10^{-8} cm/s). The data obtained in this study indicate that although lipophilicity is a main determinant in the diffusion of drug compounds across bronchial tissue, the number and position of alkyl groups also reflect the ability of the latter to cross membrane barriers.

Keywords: Permeability; Bronchial tissue; Methylxanthines; Salbutamol; Ipratropium bromide

1. Introduction

The administration of drugs via the respiratory tract has developed into a promising alternative to oral or invasive methods of drug delivery. Moreover, the therapeutic benefits of drug inhalation are well appreciated for local treatment of respiratory diseases and for systemic delivery of anesthetic agents. An important factor of systemic delivery via inhalation is the efficacious deposition of aerosolised drug to the respiratory region in the lung. This will inevitable include bronchioles and the alveoli of which the latter constitutes the optimal site for systemic absorption due to a large epithelial surface, extensive air–blood interface and thin tissue barrier.

Similar to other absorptive surfaces in the body, pulmonary absorption of drugs is generally influenced by factors such as molecular size (Enna and Schanker, 1972a), lipophilicity (Enna and Schanker, 1972b), degree of ionisation (Arakawa and Kitazawa, 1987) and various additives (Morita et al., 1993). Thus, in new drug development, studies on the relationship between the physicochemical nature and biological activity that will eventually manifest itself at a cellular level are imperative.

In attempts to better understand the factors that govern transmembrane diffusion rates of potential drug candidates, a number of biological models ranging from *in vivo* pharmacokinetic to cell culture, as well as isolated and perfused lung models have been developed (Brown and Schanker, 1983; Foster et al., 2000; Derendorf et al., 2001). In this way valuable insight about drug formulations and pharmacokinetic profiles of drugs is gained. The present study was undertaken in order to identify the permeability characteristics of bronchial tissue to various drugs by using a continuous flow-through perfusion system. For this purpose, seven alkylated xanthines, the β_2-agonist salbutamol, the quaternary antimuscarinic agent ipratropium bromide and the folate antagonist trimethoprim were used as model compounds (Fig. 1).

2. Materials and methods

Porcine bronchial tissue was obtained from the Central Research Facility and placed in a transport medium consisting of phosphate-buffered saline pH 7.4 (PBS). The study was approved by the Ethics Committee of Stellenbosch University.

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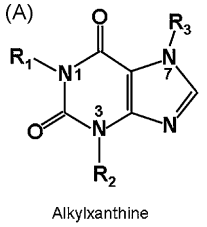
(A)



Alkylxanthine

Alkylxanthine	R_1	R_2	R_3	Mean Steady State Flux Values ($ng.cm^{-2}.min^{-1}$) ± SD
Theophylline	CH_3	CH_3	H	639.4 ± 22.6 *
Caffeine	CH_3	CH_3	CH_3	395.6 ± 6.9
Theobromine	H	CH_3	CH_3	391.6 ± 6.3
Enprofylline	H	$CH_2CH_2CH_3$	H	417.8 ± 10.9

* Estimated (18 – 24 h)

(B)

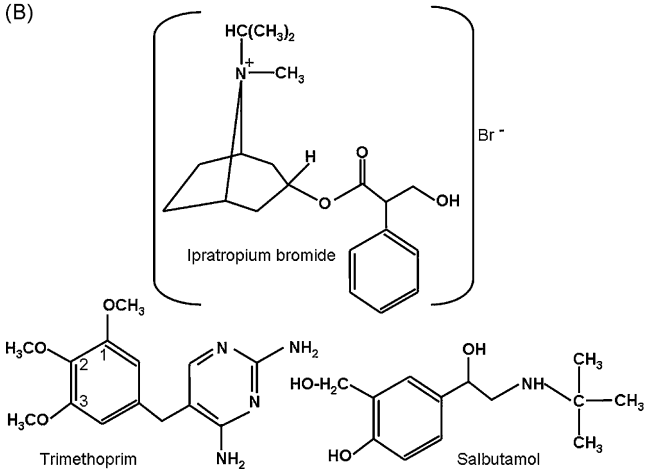


Fig. 1. Chemical structures of the studied model compounds including a table of varying alkyl substituents attached to the xanthine structure. Estimated and mean steady state flux values for the different xanthines are also reported.

2.1. Permeability experiments

The diffusion kinetics of chemical compounds through porcine bronchial tissue was determined with a flow-through diffusion system. Before each permeability experiment, tissue specimens were equilibrated for 10 min at room temperature in phosphate-buffered saline (PBS, pH 7.4). Thereafter the specimens were carefully cut, so as not to damage the epithelial surfaces, into 4 mm^2 sections and mounted in flow-through diffusion cells (exposed areas 0.039 cm^2) as previously described (Van der Bijl and van Eyk, 2003). Permeation studies were performed on 7 tissue replicates for each experiment. Before commencing each experiment, tissue disks were equilibrated for 10 min with PBS (pH 7.4) at 20 °C in both the donor and receiver compartments of the diffusion cells. After equilibration, the PBS was removed from the donor compartment and replaced with 1 ml of the drug at a concentration of 1 mg/ml. PBS at 20 °C was pumped through the receiving chambers at a rate of 1.5 ml/h and collected, by means of a fraction collector, at 2-h intervals for 24 h. The permeability study was performed under sink conditions, i.e. at the completion of each run the concentration of permeant in the acceptor chamber never reached 10% of that in the donor compartment.

2.2. HPLC detection of drugs

Permeant-containing effluent samples, collected from the acceptor compartments of the perfusion apparatus over the 2–24 h sampling intervals, were analysed using a Hewlett Packard 1100 series high-performance binary liquid chromatograph fitted with a diode-array UV detector (Agilent Technologies, Waldbron, Germany). An Agilent (SB-C_{18}) Zorbax analytical column (3.5 μm particle size), 150 mm × 4.6 mm (i.d.) was used. This column was preceded by a 30 × 4.6 mm (i.d.) SB-C_{18} guard column (3.5 μm particle size). The temperature was maintained at 40 °C, the injection volume was 20 μl and a flow rate of 1.0 ml/min was used. Run time was 5 min with a 3 min post time for column equilibration. The mobile phase consisted of a mixture of two solvents, A (50 mM KH_2PO_4, pH 5.42) and B (acetonitrile–isopropanol; 4:1, v/v). All reagents used for the mobile phase were HPLC grade. For determination of theophylline, caffeine and theobromine the isocratic mixture of A:B was as follows: 0–1 min 10% B and 1 to 5 min 45% B and detection of these compounds was at 273 nm. Enprofylline and trimethoprim were determined with an isocratic mixture of A:B as follows: 0–1 min 15% B and 1 to 5 min 50% B and were detected at 272 nm. Standard calibration curves ($R^2 = 0.999$) were constructed over the expected concentration range (1–3 μg/ml in PBS, pH 7.4) and used for quantitation of the drugs. The area under the curve of peaks obtained was used to calculate drug content of effluent samples.

2.3. LC–MS–MS analysis

LC–MS–MS analyses of ipratropium bromide and salbutamol were performed on an API 2000 Triple Quadropole (Applied Biosystems) instrument equipped with a turbulon atmospheric ionisation chamber, preceded by an Agilent 1100 series LC with autosampler. Chromatography of the samples was carried out using 0.1% formic acid in water as solvent A and methanol containing 0.1% formic acid as solvent B. Samples (5 μl) were injected into a reversed phase SB-C_{18} Zorbax analytical column (150 mm × 2.1 mm; 3.5 μm particle size) at 40 °C. Flow rate was 250 μl/min. The solvent gradient for the separation of ipratropium bromide and salbutamol is described in Tables 1 and 2, respectively. The API source was operated in the positive turbospray mode with ion source parameters as follows: Nebuliser

Table 1
Ipratropium bromide HPLC gradient conditions for separation and MS retention time, molecular- and fragmented ion

Time (min)	A (%)	B (%)	Retention time (min)	$[M+H]^+$ precursor ion (*m/z*)	Confirming product ion (*m/z*)
0–1	90	10	4.75	333	124
1–8	10	90			
8.1	90	10			
12	90	10			