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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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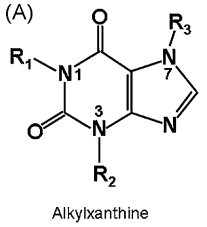
(A)



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)

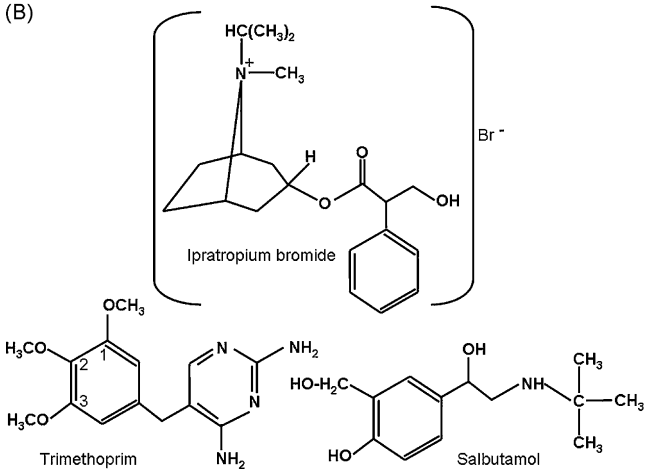


Fig. 1. Chemical structures of the studied model compounds including a table of varying alkyl substituents attached to the xanthine structure. Estimated and mean steady state flux values for the different xanthines are also reported.

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			

Table 2
Salbutamol 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*)
0–0.5	82	18	1.98	240	148
0.5–2.5	75	25			
2.5–2.55	82	18			
2.55–6.5	82	18			

gas 20; turbo gas 60; MS temperature 400 °C. Analytes were detected at a fragmentation voltage of 45 V and confirmed on the retention time of the precursor and product ions as listed in Tables 1 and 2.

2.4. Data analyses

Flux (J) values of drug compounds across bronchial tissue were calculated by means of the relationship: $J = dQ/dt \times 1/A$ ng cm^{-2} min^{-1}), where Q is the quantity of substance crossing membrane (ng), A is the membrane area exposed (cm^2), and t is the time of exposure (in min). When no statistically significant differences ($p < 0.05$) (ANOVA and Duncan's multiple range test) between flux values were obtained over at least two consecutive time intervals, a steady state (equilibrium kinetics) was assumed to have been reached for a particular drug compound. The apparent permeability coefficients, P_{app} (cm/s), were calculated by taking the initial drug concentration C_0 (1 mg/ml) into consideration and expressing the value in cm/s namely: $P_{app} = J/C_0 = dQ/dt \times 1/AC_0$. The P_{app} of each drug was plotted against the logarithm of its octanol/water distribution coefficient. $\log_{10} P$ values were calculated by using a software package from ACD/Labs (Advanced Chemistry Development, Inc., Toronto, ON, Canada M5C 1T4) as well as reference literature values when available (Rytting et al., 2005).

2.5. Statistical analyses

Non-linear regression analyses (third-order polynomial) were performed using GraphPad Prism, Version 5 (GradPad Software Inc., San Diego, CA 92130, USA). A F-test was used to compare entire curves (Motulsky, 1995). An unpaired t test with Welch's correction was used to investigate possible differences between flux means of tissues at 2-h intervals. A significance level of $p < 0.05$ was used for all tests and comparisons.

3. Results

The mean flux values of theophylline (1,3-dimethylxanthine), caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) across porcine bronchial tissue are shown in Fig. 2. Theophylline had a ~38% higher mean flux value across bronchial tissue than either caffeine or theobromine. In the 24 h period theophylline reached a steady state flux rate at ~20 h while caffeine and theobromine reached steady state after approximately 12 h.

The mean flux values found for the 4-monoalkylated xanthines, 1-methyl-, 3-methyl-, 3-propyl- and 7-methylxanthine are shown in Fig. 3. Both the 1-methyl- and 3-methylxanthines had a ~80% lower mean flux value than theophylline, whereas the flux rate of 7-methylxanthine was ~50% lower. 3-Propylxanthine reached steady state flux rate after approximately 16 h and had a ~35% lower mean flux value than theophylline.

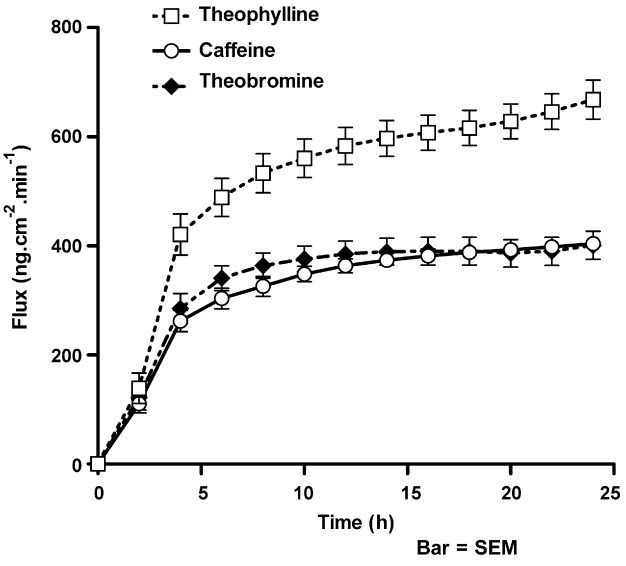


Fig. 2. Mean flux of theophylline, caffeine and theobromine across bronchial tissue.

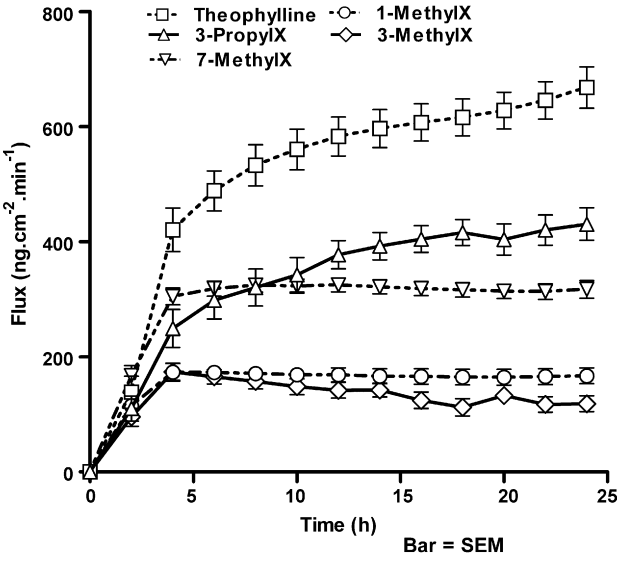


Fig. 3. Mean flux of theophylline, 1-methyl-, 3-methyl-, 3-propyl- and 7-methylxanthine across bronchial tissue.

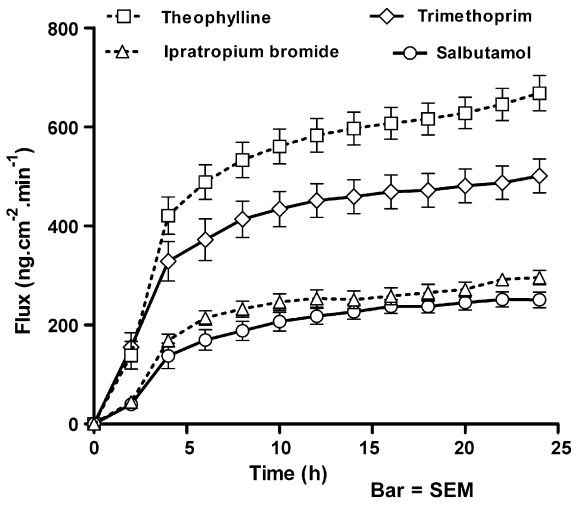


Fig. 4. Mean flux of theophylline, trimethoprim, ipratropium bromide and salbutamol across bronchial tissue.

Salbutamol ($\log_{10} P$ −0.79) and ipratropium bromide ($\log_{10} P$ −2.21) had a ∼70 and ∼64% lower mean flux value, respectively, through the bronchial tissue when compared with theophylline ($\log_{10} P$ −0.17) (Fig. 4). Both drugs reached steady state in approximately 14 h. On the other hand, the folate antagonist trimethoprim (methoxy-groups at the 1, 2 and 3 positions of the ring), had only a ∼25% lower mean flux value than theophylline and a steady state flux rate was achieved in approximately 15 h.

Fig. 5 shows the sigmoidal relationship between the apparent permeability coefficients (P_{app}) and the octanol/water partition coefficients ($\log_{10} P$) of the drugs tested. The sigmoidal curve was fitted to the data using the following equation:

$$P_{app} = Y_{min} + Y_{max} - \frac{Y_{min}}{1 + 10^{\log P50\% - \log P}}$$

where Y_{min} = minimum P_{app} and Y_{max} = maximum P_{app}.

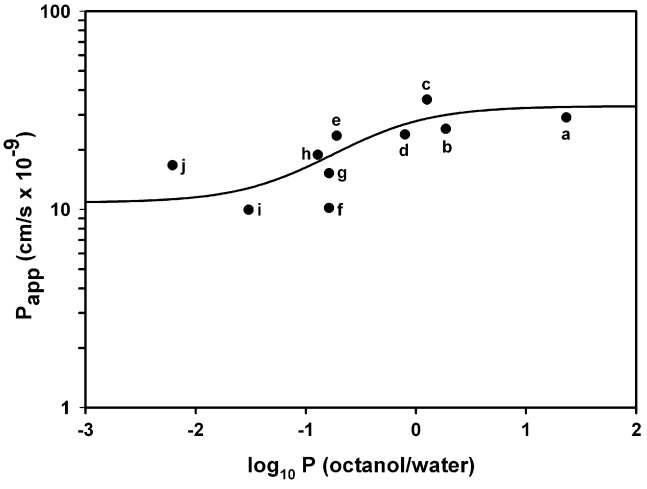


Fig. 5. Relationship between P_{app} and $\log_{10} P$ of drugs across bronchial tissue: (a) trimethoprim; (b) enprofylline; (c) theophylline; (d) caffeine; (e) theobromine; (f) 3-methylxanthine; (g) salbutamol; (h) 7-methylxanthine; (i) 1-methylxanthine; (j) ipratropium bromide.

4. Discussion

The current work has examined the relationship between chemical structure and the diffusion of various drugs across porcine bronchial tissue. With xanthine derivatives as model drugs, the number of methyl groups as well as the position of the group in the structure plays an important role in transmembrane diffusion. With the symmetrical substituted xanthine derivative theophylline (1,3-dimethylxanthine; MW 180.16), diffusion through bronchial tissue occurred with a higher flux rate than caffeine (1,3,7-trimethylxanthine; MW 194.19). We calculated that a total amount of 1.5 μg theophylline traversed 1 cm^2 the bronchial tissue per hour in comparison to a value of 0.8 μg for caffeine. Under our *in vitro* experimental conditions the uptake for theophylline (1.5 μg/cm^2/h) across porcine lung reflects the trend that it follows in the *in vivo* experiments conducted by Yamada et al. (2005) showing that it is rapidly absorbed from rat lung. A possible explanation for the much lower diffusion of caffeine might be the result of steric hindrance of the additional methyl group on position 7 of the chemical structure. On the other hand, theobromine (3,7-dimethylxanthine; MW 180.16) also had a lower flux rate similar to that of caffeine. We argue that for both caffeine and theobromine the lower flux rates could also possibly be ascribed to the increased water solubility of these xanthine derivatives, methylated at position 7 of the chemical structure (Persson, 1986). This would result in a slower distribution from the PBS into the membrane. Interestingly, theophylline's higher flux rate also coincides with its ability to act as an adenosine antagonist and augmented bronchodilator potency in comparison to caffeine. In the latter compound the extra methyl-group on position 7 leads to decreased bronchodilator potency (Persson, 1986).

It is known that lipophilicity is an important driving force sustaining passive transport of drugs across membranes. Moreover, the addition of methyl groups to the chemical structure of a drug leads to an increase in lipophilicity (Gulaboski et al., 2007). An important finding in our study is that a sigmoidal relationship exists between $\log_{10} P$ and P_{app} values of the drugs we tested. Previously a sigmoidal relationship was described in transport studies of β-blockers across alveolar epithelial cells showing a correlation between permeability and lipophilicity of drug compounds (Saha et al., 1994; Brown and Schanker, 1983). A similar trend was found in the present study despite the narrow $\log_{10} P$ range of the model drugs that we tested ($\log_{10} P$: −2.21 to 1.364), giving P_{app} values that differed only 1.7-fold. Unfortunately, there is no report in the literature on bronchial permeation of drugs to compare these results with, and the P_{app} values calculated for drugs across bronchial tissue were significantly lower than those found for alveolar and nasal cell monolayers (Lin et al., 2005). We argue that the small difference found for P_{app} may well be indicative of other contributing factors that influence permeability such as molecular weight, positioning and number of alkyl groups attached to the drug, as well as the molecular polar surface area (PSA) of the compound tested (Tronde et al., 2003). In this regard trimethoprim may serve as an example (methoxy groups at positions 1, 2 and 3 of the ring, MW 290.3 Da; $\log_{10} P$ 1.364). Although it is the most lipophilic drug that we tested, it

had a ∼25% lower flux rate through the tissue than theophylline. Another example is enprofylline (MW 194.2; log *P* 0.27), with a propyl group attached to position 3 of the xanthine structure making it more lipophilic than theophylline but less diffusable (Persson, 1986).

Bronchial epithelium, which is lipoidal in nature and consists of pseudo stratified, ciliated columnar epithelium, is the main barrier offering high resistance to the diffusion of hydrophilic and charged molecules (Mitchel et al., 1993). Our study confirms this resistance as the hydrophilic monoalkylated xanthines (MW 166.1), 1-methyl-, 3-methyl-, and 7-methylxanthine had lower flux rates than theophylline. In contrast to the intestinal mucosa (Van der Bijl and van Eyk, 2002) and the blood–brain barrier, pulmonary epithelium is highly permeable to compounds with a high PSA (Krondahl et al., 2002). In this regard ipratropium bromide with a PSA of ∼90 Å (P_{app} value of 1.6×10^{-8} cm/s) was able to diffuse across the bronchial tissue with a similar flux rate to salbutamol (P_{app} 1.5×10^{-8} cm/s) (Tronde et al., 2003). Furthermore, in a recent report the active transport of salbutamol across human airway epithelial cells was shown in two *in vitro* model systems (Ehrhardt et al., 2005).

Finally, it is important to note that bronchial epithelium differs markedly in structure to that of alveolar epithelium so that the overall drug absorption upon pulmonary administration can be very different from that observed in our *in vitro* studies with porcine bronchial tissue. However, the data of this study are considered to be a contribution to a better understanding of the predictability or correlation of drug absorption across human bronchial epithelial cell layers.

5. Conclusions

In conclusion, the present findings demonstrate the use of bronchial tissue as an *in vitro* model to evaluate the influence of the physicochemical properties of drugs on permeation in bronchial tissue. Although additional studies are needed to further elucidate chemical features favouring the development of pulmonary drugs, the findings of the present study may contribute to a better understanding of drug diffusion through the epithelium of the lower airways.

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