ELSEVIER

International Journal of Pharmaceutics 115 (1995) 95–102

international journal of pharmaceutics

Pulmonary bioavailability and absorption characteristics of the 5-lipoxygenase inhibitor, Abbott-79175, in beagle dogs

Yihong Qiu, Pramod Gupta, Howard Cheskin, Akwete Adjei *

Pharmaceutical Products Division, Abbott Laboratories, 1400 Sheridan Road, North Chicago, IL 60064–2204, USA

Received 16 June 1994; modified version received 12 August 1994; accepted 15 August 1994

Abstract

The pulmonary bioavailability and absorption characteristics of two aerosol suspensions of a potent 5-lipoxygenase inhibitor, Abbott-79175, containing different dispersants (formulations B and C) were investigated using an intravenous (i.v.) formulation of the drug as a reference. A reverse-phase HPLC assay was used to analyze drug concentrations in serial blood samples taken after drug administration to groups of nine tracheostomized dogs. Both formulations demonstrated fast onset of drug absorption from the lung which lasted for about 9 h. Data stripping yielded absolute bioavailabilities of 57.8 (±25.7) and 68.4 (±22.8)% for formulations B and C, respectively. Analysis of variance (ANOVA) yielded sources of variation in each data set thus providing a means for comparisons. Moment analysis and deconvolution (PCDCON and RSTRIP) using i.v. data as the unit impulse response enabled estimation of drug absorption from the formulations for each animal. The apparent absorption of aerosolized drug from the lungs was found to fit first-order kinetics ($k_1 = 0.083$ and 0.093 h^{-1} for formulations B and C, respectively). The results also suggested involvement of particle dissolution phenomenon as potential rate-limiting step in the absorption process (e.g., cube-root model: $k_{1/3} = 0.023$ and 0.026 h^{-1} for formulations B and C, respectively). Hence, it appears that particle dissolution as well as membrane transport may be rate determining processes in the absorption of Abbott-79175 from the airways.

Keywords: 5-Lipoxygenase inhibitor; Metered-dose inhalation; Pulmonary bioavailability; Pharmacokinetics; Moment analysis; Deconvolution

1. Introduction

Leukotrienes, 5-lipoxygenase products of arachidonic acid metabolism, are generated from a wide variety of cells in the airways, such as eosinophils, mast cells, and alveolar macrophages following immunologic stimuli (Samuelsson, 1983). 5-Lipoxygenase is an enzyme that converts arachidonic acid to 5-hydroperoxyeicosa-6,8,11, 14-tetraenoic acid (5-HPETE) in the pathway leading to the production of leukotrienes. The leukotrienes are potent mediators that can elicit many of the pathophysiologic features found in asthma, such as smooth airway muscle contraction, microvascular leakage, and chemotaxis of inflammatory cells (Drazen and Austen, 1987). These mediators are therefore thought to be im-

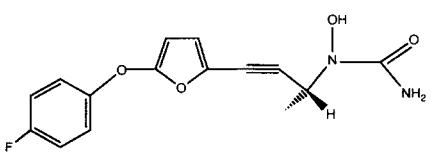
* Corresponding author.

SSDI 0378-5173(94)00259-2

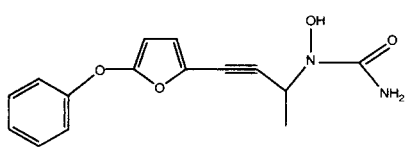
portant in the pathogenesis of asthma. The immunoscience discovery research group at Abbott is investigating a variety of inhibitors of leukotrienes as possible new chemical entities for alleviating airway muscular contraction in asthma (Hui et al., 1991).

Ovalbumin sensitized male Hartley strain guinea pigs have been shown to elicit reduced airway anaphylaxis after oral administration of 5-lipoxygenase inhibitors. The degree of inhibition of antigen-induced airway anaphylaxis following oral administration of 5-lipoxygenase inhibitors is dose dependent. Furthermore, the degree of inhibition of the antigen-induced airway anaphylaxis is directly proportional to plasma drug concentration and recoverable amounts of the drug in bronchoalveolar lavage fluids (Malo, 1990). One of these compounds, Abbott-79175, chemically known as *R*-(+)-*N*-[3-[5-(4-fluorophenoxy)-2-furanyl]-1-methyl-2-propynyl]-*N*-hydroxyurea, is a potent 5-lipoxygenase inhibitor currently under investigation as a new drug for the treatment of allergic airway diseases. The chemical structures for Abbott-79175 and the internal standard used in the analytical method, Abbott-78734, are shown in Scheme 1.

Inhibition of leukotriene synthesis has many potential therapeutic benefits for conditions in which leukotriene synthesis is elevated, such as asthma. Although 5-lipoxygenase inhibitor compounds are orally active, their inhalation delivery to the lungs was hypothesized to offer significant reduction in the clinically effective dose and thus improve its safety. Hence, studies were undertaken to develop Abbott-79175 metered dose inhaler (MDI) formulations. The objectives of these studies were: (a) to evaluate absolute bioavailability of two test aerosol formulations containing different dispersants using i.v. administration as a reference standard, and (b) to study the pulmonary absorption characteristics of Abbott-79175 using linear system analysis.



A-79175



A-78734

Scheme 1. Chemical structure of 5-lipoxygenase inhibitors.

2. Materials and methods

2.1. Materials and equipment

The following materials were used in the study: Abbott-79175, lot no. 64-225-AL and Abbott-78734, internal standard, lot no. 128747-0 (Pharmaceutical Products Division, Abbott Laboratories); and tetrafluoroethane, HFA-134a (E.I. DuPont de Nemours & Co.). All other chemicals and reagents were either AR or HPLC grades and used as received. A Malvern laser particle size analyzer, series 2600c, with spray synchronizer (model PS51), infrared sensor (model PS57) and rotation trigger sensor (model PS58) was used to determine the particle size distribution of the inhalation aerosols. An HPLC system consisting of a Spectra Physics HPLC pump and auto sampler, a Kratos Analytical Spectraflow 783 UV detector, and a Spectra Physics integrator were used for sample assays.

2.2. Formulations

Two suspension MDI formulations, B and C, each containing 10 mg/ml Abbott-79175 in HFA-134a were prepared and used in the study. The reference product, formulation A, was a 2 mg/ml intravenous (i.v.) solution of Abbott-79175 containing water/ethanol/propylene glycol (50:25:25% v/v) as a solvent.

2.3. Primary packaging and product stability

The primary package for the MDI formulations consisted of a 100 μl metering valve

(DF10/RC, Valois, Le Neuborg, France) and a 20 ml epoxy phenolic lined aluminum container (Safet Embamet, St. Florantine, France). Functional performance tests (shot weight and content uniformity) were conducted to ensure dose reproducibility. Agglomeration was evaluated by visual observation and monitoring particle size distribution in the suspension. Valve performance was examined by measuring dose of drug delivered per spray as a function of time and storage conditions. The stability of formulations was found to be satisfactory for at least 1 month after the initiation of the bioavailability studies. An actuator device (Micron-4, Abbott Laboratories) was modified by removal of the main plastic body to enable stem insertion into dog trachea and deliver the dose.

2.4. In vivo studies

Nine tracheostomized beagle dogs weighing 7.9–9.5 kg were used in the study. A three-way cross-over design with 1-week dosing intervals was used to study the two MDI formulations against the i.v. solution. The dose of i.v. solution was adjusted such that the dogs received 0.5 mg/kg drug. Five sprays of each aerosol formulation were delivered to each dog. Each spray contained a nominal dose of 1 mg Abbott-79175. This translated to actual inhalation drug doses of 0.53–0.67 mg/kg. Serial blood samples were collected over 24 h following dose administration and stored frozen at −20°C until assayed for drug concentration by HPLC. The data following inhalation drug delivery was normalized to a dose of 0.5 mg/kg.

2.5. Sample processing and analytical procedure

A 0.1 ml aliquot of the internal standard solution was combined with 0.2 ml blood sample and then mixed with 6 ml methylene chloride/ethanol solution (9:1, v/v). The resulting mixture was shaken at low speed and centrifuged. The organic phase was transferred and evaporated to dryness. The dried samples were reconstituted with 0.30 ml of methanol/water (3:7 v/v) for assay. The HPLC system included a Regis Little Champ C-18 column (50 × 4.6 mm, Spherisorb, 3 μm) and a mobile phase consisting of tetrahydrofuran/aqueous solution (1:3, v/v) at a flow rate of 1.0 ml/min. The aqueous solution contained 0.13% tetramethylammonium perchlorate and 0.075% trifluoroacetic acid. The UV detection wavelength was 260 nm and Abbott-78734 was used as an internal standard. For each set of test blood samples, a calibration curve was constructed with spiked standards.

2.6. Data analysis

The area under the blood concentration-time curve (AUC) from time zero to the last sampling time point t (AUC_t) was calculated according to the trapezoidal rule. AUCs from time zero to time infinity (AUC_∞) were obtained by extrapolation using elimination rate constants from intravenous administration. For the inhalation treatments, the AUCs were normalized on the basis of dog weights. Peak drug concentration (C_{max}) and the time to peak drug concentration (T_{max}) were obtained directly from the data without interpolation. Analysis of variance (ANOVA) was performed on the parameter AUC_∞ using JMP 2.0.5 (SAS Institute, Inc., Cary, NC). The sources of variation included in the model were formulation, subjects, sequence and period. The 90% confidence interval for the ratio between the test and reference average AUC_∞ was used to test the two one-sided hypotheses at a significance level of 0.05.

3. Results and discussion

3.1. Formulation particle size and stability

Fig. 1 compares the ex-valve particle size range of the two inhalation aerosols tested in this study. With both formulations about 28% of the particles were in the respirable range, i.e., < 5.3 μm, and 50% of the emitted particles were under 7.7 μm. For formulations B and C, 44 and 37% of the particles were in the range of 5.3–9.6 μm, respectively. The respective mean particle diameters for both formulations were statistically non-

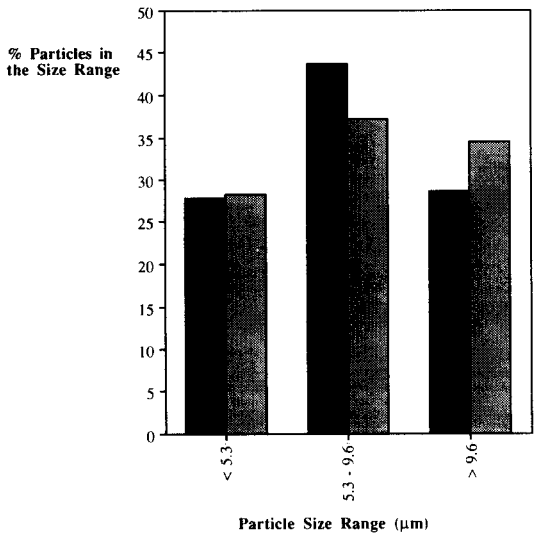


Fig. 1. Particle size of two inhalation formulations tested in the study. (Hatched bars) MDI formulation B; (stippled bars) MDI formulation C.

significant ($p > 0.05$) from each other. Since almost 30% of the particles in both formulations were greater than 9.6 μm, these formulations need further optimization from process standpoint to become suitable for lung delivery. Nonetheless, the formulations were deemed acceptable to assess the effect of dispersants on the Abbott-79175 absorption kinetics and bioavailability from the lungs. Chemical stability studies revealed no measurable losses in Abbott-79175 concentrations for the duration of the study.

3.2. Shot weight and drug content uniformity

Gravimetric measurements of serially actuated shots of a test MDI formulation provide a quick indication of its shot-to-shot variation. This method aids in the estimation of dose uniformity. Mean shot weight data indicated no difference ($p > 0.05$) in the performance of the two MDI formulations. 'Through-life' valve delivery as a function of dosing sequence was conducted to simulate field-use conditions. Statistical analysis of mean shot weight results for the two formulations did not show any measurable trends or significant differences ($p > 0.05$) in dosimetry of the product. The respective mean shot weights were well within current compendial limits of ±15% of the nominal dose.

3.3. Bioavailability

The whole blood concentration profiles of Abbott-79175 following single dose administration of the three formulations to beagle dogs are shown in Fig. 2. The mean pharmacokinetic parameter values for the three treatments are summarized in Table 1. For formulations B and C, the estimates of absolute bioavailability were 57.8

Table 1
Absolute bioavailability and pharmacokinetic parameters of Abbott-79175 following inhalation delivery to dogs ($n = 9$)

Parameter	i.v.	MDI B	MDI C
AUC_{24} (h μg ml^{-1})	12.27 (±1.40)	5.70 (±1.99)	6.48 (±2.19)
AUC_{∞} (h μg ml^{-1})	13.04 (±1.82)	7.45 (±3.13)	8.81 (±3.01)
C_{max} (μg ml^{-1})	–	0.56 (±0.13)	0.55 (±0.12)
T_{max} (h)	–	5.03 (±3.83)	6.78 (±2.91)
F [a]	1.00 (±0.00)	0.58 (±0.26)	0.68 (±0.23)
90% CI [b]	–	(0.38–0.78)	(0.51–0.86)
MRT (h)	9.35 (±1.80)	13.39 (±1.90)	13.13 (±1.91)
MAT (h)	–	3.70 (±2.10)	3.60 (±1.69)
k (h^{-1})	0.120 (±0.017)		
$t_{1/2}$ (h)	5.89 (±0.85)		
Cl (l h^{-1} kg^{-1})	0.04 (±0.004)		
V (l kg^{-1})	0.33 (±0.03)		

[a] Based on AUC_{∞}.
[b] 90% confidence intervals for the ratio of the test and reference formulation.

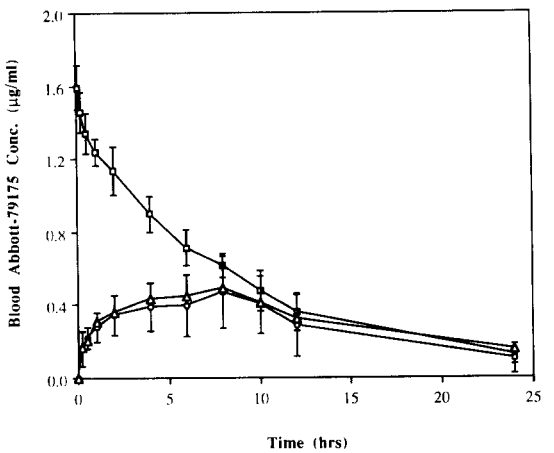


Fig. 2. Mean blood concentration-time profiles following i.v. and inhalation delivery of 0.5 mg/kg of Abbott-79175 to beagle dogs ($n = 9$) (□) i.v.; (○) MDI formulation B; (△) MDI formulation C. Each point represents the mean ± standard deviation for nine dogs.

(±25.7) and 68.4 (±22.8)%, respectively. Both aerosol formulations demonstrated fast onset of drug absorption which lasted for up to 9 h. Based on ANOVA, formulations ($p < 0.001$) and period ($p = 0.044$) were found to be the main sources of variation. The effects of subject ($p = 0.082$) and sequence ($p = 0.763$) were insignificant.

3.4. Pharmacokinetics

A non-linear least-squares program RSTRIP II (MicroMath® Scientific Software, Salt Lake City) was used to fit the blood concentration data from individual animals to polyexponential equations. The goodness of fit was assessed based on model selection criteria (MSC), a modified Akaike information criterion (AIC), and examination of parameter redundancy. In most cases, a good fit to a single exponential equation was obtained for the data following the i.v. administration of Abbott-79175. Improved fits to two-exponentials were observed for the data from dogs 2, 5 and 9. The average elimination half-life of Abbott-79175 was found to be 5.88 (±0.85) h. However, none of the blood concentration data following inhalation drug delivery could be fitted to two or three exponentials. The blood-concentration data were used to estimate the in vivo mean residence time (MRT) of systemically and pulmonary-delivered drug, and mean absorption time (MAT) of aerosolized drug, according to the equations (Veng-Pedersen, 1989):

$$\mathrm{MRT} = \frac{\int_0^\infty tC(t)\,\mathrm{d}t}{\int_0^\infty C(t)\,\mathrm{d}t} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \quad (1)$$

$$\mathrm{MAT}_{\mathrm{MDI}} = \mathrm{MRT}_{\mathrm{MDI}} - \mathrm{MRT}_{\mathrm{i.v.}} \quad (2)$$

where AUC and AUMC are areas under the blood concentration [$C(t)$] and the first moment curves, respectively. For the two aerosol formulations, both AUC and AUMC were calculated based on the trapezoidal rule. Integrals of AUMC from the last time point (t_n) to infinity were estimated using $\mathrm{AUMC}_{t_n-\infty} = t_n \cdot C_n/k + C_n/k^2$, where k is the terminal rate constant following i.v. drug administration. $\mathrm{MRT}_{\mathrm{i.v.}}$ represents the mean residence time of the drug in the systemic circulation after i.v. administration. $\mathrm{MAT}_{\mathrm{MDI}}$ refers to mean time involved in the in vivo dissolution and absorption of drug from the lung. The average $\mathrm{MRT}_{\mathrm{i.v.}}$ was estimated to be 9.53 (±1.80) h. The average MATs for formulations B and C were 3.70 (±2.10) and 3.60 (±1.69) h, respectively.

3.5. Absorption analysis

Linear system analysis is a model independent method useful in the evaluation of drug absorption processes. Based on the superposition principle in a linear time-invariant system (Cutler, 1978a), a response, $C(t)$, to an input, $f(t)$, of the system can be obtained using the following convolution integral:

$$C(t) = f(t) * C_\delta(t) = \int_0^\infty C_\delta(t-\tau) f(\tau)\,\mathrm{d}\tau \quad (3)$$

where $C_\delta(t)$ is the unit impulse response characteristic of the system. For most pharmacokinetic applications, $C(t)$ and $f(t)$ represent the blood drug concentration and the rate at which drug enters the system, respectively (Cutler, 1978a). $C_\delta(t)$ is the blood concentration resulting from

the instantaneous input of a unit amount of drug into the system.

In the present study, individual blood concentration profiles following i.v. administration were used as unit impulse responses $C_\delta(t)$. Drug blood data from the aerosol formulations, $C(t)$, were fitted to a smoothing cubic spline function and then deconvoluted with $C_\delta(t)$ using the Program PCDCON (provided by Dr W. Gillespie) to obtain apparent in vivo drug absorption profiles (Fig. 3). The fractional absorption, as estimated from the plateau values of both profiles, closely matched the absolute bioavailability based on the AUCs of the two formulations, i.e., 0.58 vs 0.59 for B, and 0.68 vs 0.63 for C. The average in vivo absorption profiles of Abbott-79175 from both aerosol formulations approximated first-order kinetics which lasted for about 9 h.

Following deposition of the delivered drug particles, apparent drug absorption from the lung primarily involves in vivo particle dissolution followed by diffusion or pinocytosis into the systemic circulation (Ganderton and Jones, 1987). Therefore, the kinetics of apparent drug absorption depends upon the rate-limiting process. Passive diffusion is the most common mechanism of drug transport through biological membranes (Abdou, 1989). For drugs with low aqueous solubility, like Abbott-79175 which has an aqueous solubility of ~ 15 μg/ml, absorption may indeed also depend on particle dissolution characteristics. In order to characterize the absorption processes, the averaged in vivo absorption profiles were fitted to first-order kinetics and three particle dissolution models: Hixson-Crowell (i.e., cube-root), Higuchi-Hiestand (i.e., 2/3-root) and

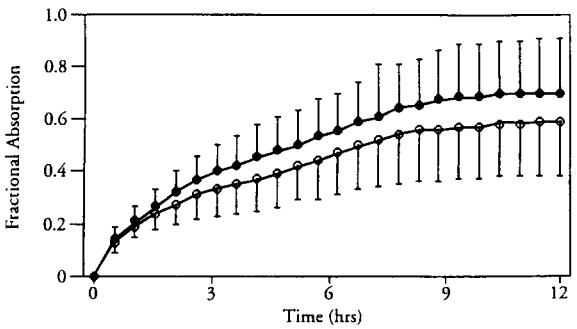


Fig. 3. In vivo absorption of Abbott-79175 estimated by deconvolution after inhalation delivery to dogs ($n = 9$). (○) MDI formulation B; (●) formulation C.

Niebergall-Goyan (i.e., square-root) models (see Table 2 and Fig. 4) (Abdou, 1989). The fact that first-order as well as cube-root models resulted in a good fit suggests that particle dissolution as well as membrane transport may be rate-limiting in the absorption of Abbott-79175 from the lungs. The apparent first-order process may be related to absorption of the drug from the respiratory zone of the lung (i.e., transport across tight junctions of alveolar cells). The cube-root model thus should be a result of particle dissolution process in the transitional zones of the lung. However, this is a hypothesis. Indeed, it is also possible that prolonged absorption of this hydrophobic compound is a result of its deposition in regions of lung with comparatively low transport rates and/or its bindings to components of the lung tissue. Further studies are needed to fully understand the mechanism of absorption of this drug from the lungs.

Table 2
Fitting of in vivo absorption of Abbott-79175 to different models following inhalation delivery to dogs ($n = 9$)

Theoretical model	MDI formulation B			MDI formulation C		
	K_j [a]	I [b]	R^2	K_j [a]	I [b]	R^2
$(1 - X(t))^{1/2}$	0.032	0.927	0.9886	0.036	0.925	0.9847
$(1 - X(t))^{1/3}$	0.023	0.954	0.9941	0.026	0.954	0.9847
$(1 - X(t))^{2/3}$	0.041	0.904	0.9872	0.044	0.902	0.9827
$\ln(1 - X(t))$	0.083	−0.127	0.9945	0.093	−0.127	0.9951

[a] K_j represents $K_{1/2}$, $K_{1/3}$, $K_{2/3}$, or K_1.
[b] I denotes the intercept from the regression equation.

Polynomials are usually capable of approximating a wide range of functions. To a lesser extent, this approach depends on assumptions concerning the functional forms of the input (Cutler, 1978b). The exponential input functions and those derived from the cube-root dissolution model with added noise can be represented by polynomials (Cutler, 1978b). As the degree of the polynomial increases, the approximations representing the data error improve. Therefore, the apparent drug absorption profiles with both MDI formulations were fitted to different degrees of polynomials using the JMP 2.0.5 program (SAS Institute, Inc., Cary, NC). The goodness of fit was judged based on coefficients of determination as well as the t ratio of parameter estimates. The final degree of the polynomial was selected at a point when the inclusion of an additional term did not result in a significant difference. The results demonstrated best fit by quadratic polynomials for data with MDI formulations B and C. The equations describing the fit were:

$$X_B(t) = 0.236t\ (p < 0.001) - 0.070t^2\ (p < 0.001) + 0.011t^3\ (p < 0.001) - 0.001t^4\ (p < 0.001)$$
$$R^2 = 0.9984 \quad (4)$$

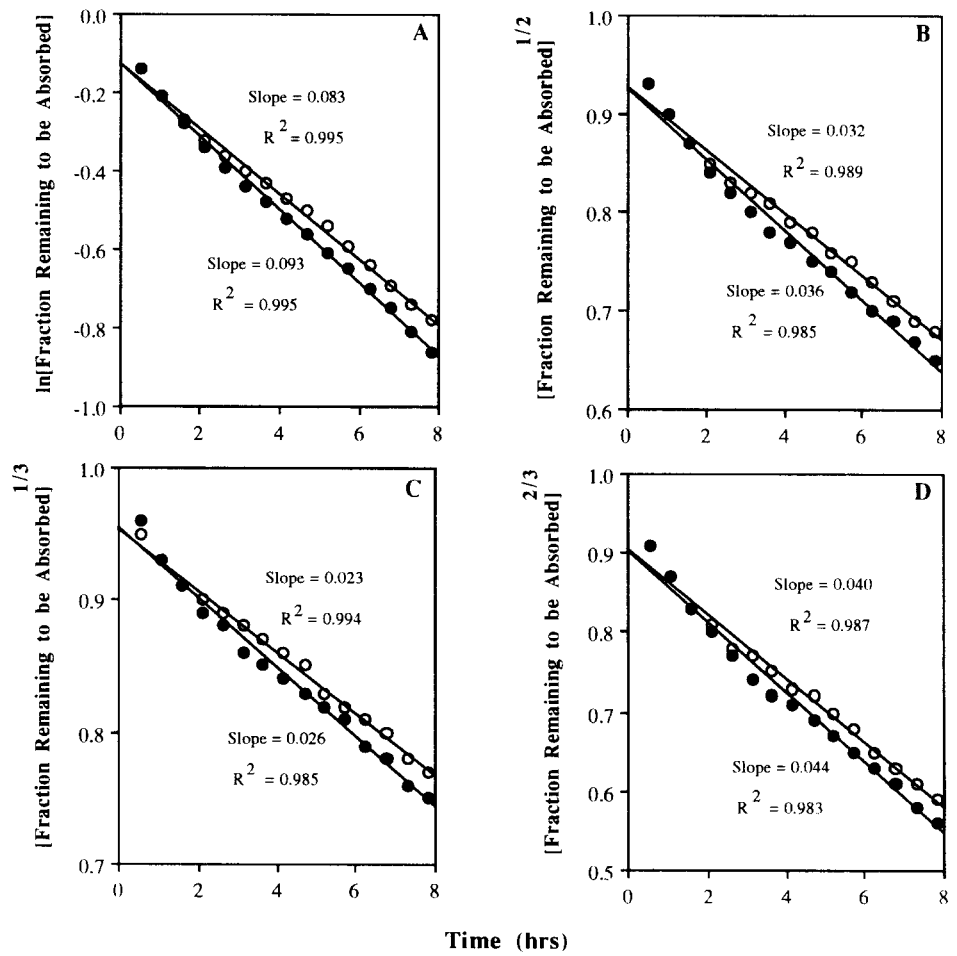


Fig. 4. Model fitting of average absorption profiles after inhalation delivery of Abbott-79175 via MDI formulations to dogs. (○) MDI formulation B; (●) formulation C. (A) First-order model; (B) square-root model; (C) cube-root model; (D) 2/3-root model.

$$X_C(t) = 0.222t\ (p < 0.001) - 0.054t^2\ (p < 0.001) + 0.007t^3\ (p < 0.001) - 0.0003t^4\ (p = 0.003)$$

$$R^2 = 0.9984 \quad (5)$$

where $X_B(t)$ and $X_C(t)$ represent the amount of drug absorbed at time t for MDI formulations B and C, respectively. In all cases, p values ≤ 0.01 were obtained for test hypotheses concerning the importance of each parameter estimate adjusted for all the other parameters in the model.

4. Conclusions

The inhalation delivery of Abbott-79175 formulated in alternate propellant HFA-134a demonstrated rapid onset and prolonged absorption of drug with bioavailability approximating 60%. This figure has not been corrected for non-absorptive losses of drug, i.e., drug lost in the device or exhaled with breath. Following correction for these losses, the blood levels and bioavailability of drug with the two inhalation formulations may be more favorable than the orally administered drug. The high pulmonary bioavailability of Abbott-79175 observed in this study may be in part attributed to the dosing methodology used, i.e., spraying drug directly into the trachea. This suggests a comparable dosing efficiency would be unachievable for oral inhalation since $\geq 75\%$ drug is presumably lost by impaction in the device, throat, and exhalation with the breath (Gupta and Hickey, 1991).

Apart from pulmonary bioavailability, the use of tracheostomized dog as an animal model enabled assessment of the drug absorption mechanism from the lungs. The results suggested that particle dissolution as well as membrane transport may be rate determining processes in the absorption of Abbott-79175 from the airways. In view of the avoidance of hepatic first pass clearance, improved stability of this drug in the airways, and good absorption from the lungs, the results suggest that the inhalation delivery of 5-lipoxygenase inhibitors is feasible and that it may provide a rational alternative regimen for the treatment of asthma.

Acknowledgments

Technical assistance from C. Allexon, D. Lee, T. Barber, L. Ruiz and D. Carpenter is appreciated. The authors also would like to thank Dr W. Gillespie for providing the PCDCON program.

References

Abdou, H., *Dissolution, Bioavailability and Bioequivalence*, Mack, Easton, PA, 1989, p. 308.

Cutler, D., Linear system analysis in pharmacokinetics. *J. Pharmacokinet. Biopharm.*, 6 (1978a) 265–282.

Cutler, D., Numerical deconvolution by least squares: Use of polynomials to represent the input function. *J. Pharmacokinet. Biopharm.*, 6 (1978b) 243–263.

Drazen, J.M. and Austen, K.F., Leukotrienes and airway response. *Am. Rev. Respir. Dis.*, 136 (1987) 985–988.

Ganderton, D and Jones, T., Drug delivery to the respiratory tract. In *Biomedicine*, Ch. 1, Ellis Horwood, Chichester, 1987.

Gupta, P.K. and Hickey, A.J., Contemporary approahes in aerosolized drug delivery to the lung. *J. Controlled Release*, 17 (1991) 129–148.

Hui, K.P., Taylor, I.K., Taylor, G.W., Rubin, P., Kesterson, J., Barnes, N.C. and Barnes, P.J., Effect of a 5-lipoxygenase inhibitor on leukotriene challenge in asthmatic patients. *Thorax*, 46 (1991) 184–189.

Malo, P., Abbott Laboratories, North Chicago, IL. Personal correspondence. *Aerosol Formulation Update*, 1990.

Samuelsson, B., Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*, 200 (1983) 568–575.

Veng-Pedersen, P., Mean time parameters in pharmacokinetics. *Clin. Pharmacokinet.*, 17 (1989) 345–366.