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Pulmonary delivery of the 5-lipoxygenase inhibitor, Abbott-85761, in beagle dogs

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Abstract

Abbott-85761 is a potent 5-lipoxygenase inhibitor which is currently under investigation for the treatment of asthma. With a primary objective of targeting this compound to the lung and reducing systemic exposure, studies were undertaken to assess its lung delivery from a metered-dose inhalation (MDI) aerosol. Pulmonary absorption characteristics of Abbott-85761 from an intratracheally (I.T.) instilled aqueous solution at doses of 0.25, 0.50 and 0.75 mg/kg were ascertained. Using tracheostomized beagle dogs, and a parallel three-way crossover study design, the I.T. results were compared with equivalent doses of intravenously (I.V.) administered drug. Plasma drug concentrations were analyzed using a reverse-phase HPLC assay. Despite limited aqueous solubility, Abbott-85761 was rapidly absorbed from the lung after I.T. instillation, with complete absorption occurring within 15–30 min after dosing. A linear dose proportionality as a function of AUC was observed for I.V. as well as I.T. treatments, with pulmonary bioavailability approximating 80%. The MDI formulation contained 10 mg/ml drug in tetrafluoroethane (HFC-134a). In vitro tests for functional performance (dispersion quality, dose delivery, content uniformity and particle size) revealed a homogeneous, physically stable and a nearly monodispersed formulation. In vivo bioavailability studies were thus conducted using a three-way crossover study design, evaluating 0.5 mg/kg aerosolized Abbott-85761 and equivalent oral and I.V. dosages of the drug. Results demonstrated that the aerosol formulation was slowly absorbed from the lung. Plasma T_{max} was approximately 7 h with pulmonary bioavailability about 40% compared to I.V. administration. The results are discussed in the context of formulation effects and delivery technique on pulmonary absorption of A-85761. © 1997 Elsevier Science B.V.

Keywords: 5-lipoxygenase inhibitors; Abbott-85761; Metered-dose inhalation; Pulmonary absorption; Pulmonary bioavailability; Pharmacokinetics of inhaled drugs; Moment analysis

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1. Introduction

Abbott-85761 is a potent second generation 5-lipoxygenase inhibitor which has been found to be effective in the manangement of asthma. It is more potent and has a longer biological half-life than its predecessor, Zileuton. Like other 5-lipoxygenase inhibitors (Fig. 1), this compound is orally bioavailable. However, at orally effective clinical doses, it has the potential to transiently stimulate liver enzymes. Therefore, it was hypothesized that lung delivery of Abbott-85761, could be clinically and commercially attractive possibly due to lower therapeutic doses as well as sustained drug output from the aerosol (Gupta and Hickey, 1991; Gupta and Adjei, 1997a). Earlier studies demonstrated that aerosol delivery of Abbott-79175 yields prolonged drug retention in the lung as well as sustained concentrations of the parent drug in plasma (Qiu et al., 1995; Adjei et al., 1996).

Preformulation studies indicated limited aqueous solubility of Abbott-85761 (~ 3.6 μg/ml) compared with Abbott-79175 (~ 17 μg/ml) and Abbott-64077 (~ 170 μg/ml) (Data on file, Abbott Laboratories). Hence, it was deemed necessary to examine absorption characteristics of Abbott-85761 from the lungs and to investigate dose effects on absorption of this drug following administration by this route. The objectives of the present study were: (1) to study the absorption characteristics of Abbott-85761 from the lung; (2) to evaluate absolute bioavailability and dose proportionality of lung instilled solution formulations of Abbott-85761 using intravenous (I.V.) drug administrations as control; (3) to develop a metered dose inhalation (MDI) aerosol of Abbott-85761 in a non-chlorofluorocarbon propellant, HFC-134a; and (4) to assess the pulmonary absorption and bioavailability of aerosolized drug.

2. Materials and methods

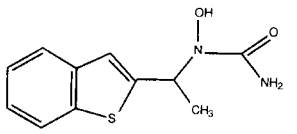
2.1. Materials and equipment

The following materials were used in the study: Abbott-85761, lot no. 75-502-VF, 79–590-AL and 77-559-AL and internal standard, Abbott-86531, lot no. 142221 (Pharmaceutical Products Division, Abbott Laboratories); and tetrafluoroethane, HFC-134a (E.I. DuPont de Nemours). 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 aerosol formulations. 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 drug assays.

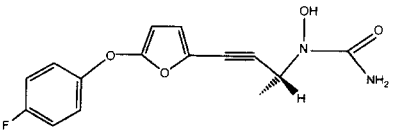
2.2. Effect of dose on intratracheal absorption of Abbott-85761

2.2.1. Formulations

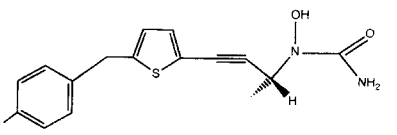
Three solutions containing 2.5, 5.0 and 7.5 mg/ml Abbott-85761 were prepared by dissolving the drug in a mixed solvent of PEG 400–water–ethanol (2:1:1) immediately before dosing.



Abbott-64077 (Zileuton)
R,S(±) N-(1-(benzo-[b]-thiene-2-yl)ethyl)-N-hydroxyurea
Molecular formula: $C_{11}H_{12}N_2O_2S$; Molecular weight: 236.29



Abbott-79175
R(+) N-[3-[5-(4-Fluorophenoxy)-2-furanyl]-1-methyl-2-propynyl]-N-hydroxyurea
Molecular formula: $C_{15}H_{13}FN_2O_4$; Molecular weight: 304.28



Abbott-85761
R(+) N-[3-[5-(4-Fluorophenoxy)-thiophen-2-yl]-1-methyl-2-propynyl]-N-hydroxyurea
Molecular formula: $C_{16}H_{15}FN_2O_2S$; Molecular weight: 318.37

Fig. 1. Chemical structure of 5-lipoxygenase inhibitors.

Table 1
Protocol for the dog study evaluating the effect of dose on the pulmonary bioavailability of Abbott-85761

Group	Dog no.	Route of administration	Week 1 (mg/kg)	Week 2 (mg/kg)	Week 3 (mg/kg)
A	1	I.T.	0.20	0.50	0.75
	2	I.T.	0.50	0.75	0.25
	3	I.T.	0.75	0.25	0.50
	4	I.T.	0.25	0.50	0.75
	5	I.T.	0.50	0.75	0.25
	6	I.T.	0.75	0.25	0.50
B	1	I.V.	0.25	0.50	0.75
	2	I.V.	0.50	0.75	0.25
	3	I.V.	0.75	0.25	0.50

2.2.2. In vivo studies

Nine tracheostomized beagle dogs weighing 8.9–10.6 kg were randomly divided into two groups. Groups I and II contained six and three dogs, respectively. A three-way crossover design with a 1-week wash-out period was used for both groups of dogs (Table 1). Overall, six dogs received each dose via the lung and three dogs received each dose I.V. on the same day. Drug solution (1 ml) was instilled into the trachea of each dog in group I. The same solution was administered I.V. to each dog in group II. This translated to doses of 0.25, 0.5 and 0.75 mg/kg. Due to the low aqueous solubility of Abbott-85761, the reference formulation was infused I.V. over a 1 min period. Serial blood samples were collected at 0, 0.25, 0.5, 1, 2, 3.5, 5, 7, 9, 12 and 24 h after single intratracheal (I.T.) instillation, and at 0, 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h following I.V. administration. The plasma samples were separated, kept frozen at -20°C for ≤ 1 week, and assayed for drug concentration. This storage condition was found to have no impact on the stability of samples. The data were normalized for differences in dog weight based on the nominal dosages of 0.25, 0.5 or 0.75 mg/kg.

2.2.3. Sample processing and drug analysis

A 0.5 ml blood sample was combined with 0.1 ml of the internal standard (Abbott-86531) solution, which was then mixed with 6 ml of a solution containing methylene chloride:ethanol (9:1 v/v). The resulting mixture was shaken at low speed for approximately 20 min Following centrifugation at 2500 rpm for 10 min, the aqueous layer was aspirated to waste. The organic phase was transferred to a test-tube and evaporated to dryness with a gentle stream of dry air over low heat (< 45°C). The sample was reconstituted with 0.30 ml of methanol:water (3:7 v/v) for HPLC analysis.

A reversed-phase HPLC assay was used to determine the concentration of Abbott-85761 in the plasma samples. A Regis Little Champ C-18 column (50×4.6 mm, Spherisorb, 3 μm) was used for the assay. The mobile phase consisted of tetrahydrofuran:aqueous solution (25:75 v/v). The aqueous solution contained 0.13% tetramethylammonium perchlorate and 0.075% trifluoroacetic acid. The UV detection and flow rate were set at 260 nm and 1.0 ml/min, respectively.

For each set of blood samples, a calibration curve (0.05–2.0 μg/ml) was constructed with spiked standards and used for calculation of the sample concentrations. Selectivity was assessed by examining peak interference from endogenous matrix components. The within- and between-day variability with the analytical technique was $\leq$ 6%.

2.3. Pulmonary absorption of aerosolized Abbott-85761

2.3.1. Formulation

A suspension MDI aerosol containing 10 mg/ml Abbott-85761 in HFC-134a was prepared and used in the study. The reference products for oral and I.V. administration consisted of 0.25 and 1

mg/ml drug solutions, respectively, in 50–60% v/v polyethylene glycol 400 in water.

2.3.2. Primary packaging, dosimetry and stability of MDI formulation

Primary packaging for the MDI formulation consisted of a 50 μl metering valve (DF10/RC, Valois, France) and a 20 ml epoxy phenolic lined aluminum container (Safet Embamet, France).

Functional performance tests (shot weight and content uniformity) were conducted to insure dose reproducibility. Valve performance was examined by measuring dose of drug delivered per spray as a function of time and storage conditions. For shot weight testing, the formulation was primed by spraying five times to waste and then weighed. Thereafter, five consecutive sprays were made in air (fume-hood) and the aerosol container re-weighed to determine the mass of the sprays. The process was repeated until all containers were empty (tail-off), and the weight of set of consecutive five sprays recorded. To monitor drug content uniformity after priming, the formulation was sprayed five times while immersing the valve stem in a beaker containing ethanol. The contents of the solution were gently swirled to solubilize the drug. The resulting solution was diluted with methanol–water solution (1:1) and assayed for drug content using the HPLC method described above. Agglomeration was evaluated by visual observation and monitoring particle size distribution in the suspension using the Malvern Model 2600c.

Chemical stability performance of the formulation was satisfactory for at least 1 month after the initiation of the bioavailability studies.

2.3.3. In vivo studies

A three-way crossover study was conducted in nine tracheostomized dogs to compare the pulmonary absorption and plasma distribution of aerosolized drug with orally and I.V. administered controls. Each formulation for oral and I.V. delivery was prepared immediately prior to use. In each stage of the study, groups of three dogs received 0.5 mg/kg Abbott-85761 in one of the following forms:

(a) Ten sprays of 0.5 mg A-85761/spray from MDI system utilizing an actuator device (Micron-4, Abbott Laboratories) with the mouth adapter and expansion chamber removed to enable insertion of the spray jet into the tracheal stoma of each dog.

(b) 20 ml of 0.25 mg/ml Abbott-85761 solution containing 50% v/v polyethylene glycol 400 in water. This solution was orally administered to each dog.

(c) 5 ml of 1 mg/ml Abbott-85761 solution containing 60% v/v polyethylene glycol 400 in water. This solution was administered I.V. over a period of 1–2 min to each dog.

Blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after dosing and stored frozen at −20°C until assayed for drug concentration using the HPLC method described above. The data were normalized to a dose of 0.5 mg/kg to account for differences in dog weight.

2.4. Data analysis

The area under the blood concentration-time course curve (AUC) from time zero to the last sampling time point t (AUC_t) was calculated by trapezoidal approximation. The AUC's were normalized to the respective doses on the basis of dog weights. Peak drug concentration (C_{max}) and the time taken for the peak blood concentration to occur (T_{max}) were obtained directly from the raw data. Analysis of variance (ANOVA) was performed on the parameter AUC_∞ using JMP 2.0.5 (SAS Institute, Cary, NC). The sources of variation included in the model were formulation dose (or route of formulation administration), subjects, sequence and/or period. The 90% confidence interval for the ratio between the test (i.e. I.T. treatment) and reference (i.e. I.V. treatment) average AUC_{24} was used to test the two one-sided hypotheses at a significance level of 0.05.

3. Results and discussion

3.1. Effect of dose on intratracheal absorption of Abbott-85761

Fig. 2 displays the mean blood concentrations following single I.V. administration of 0.25, 0.5 or

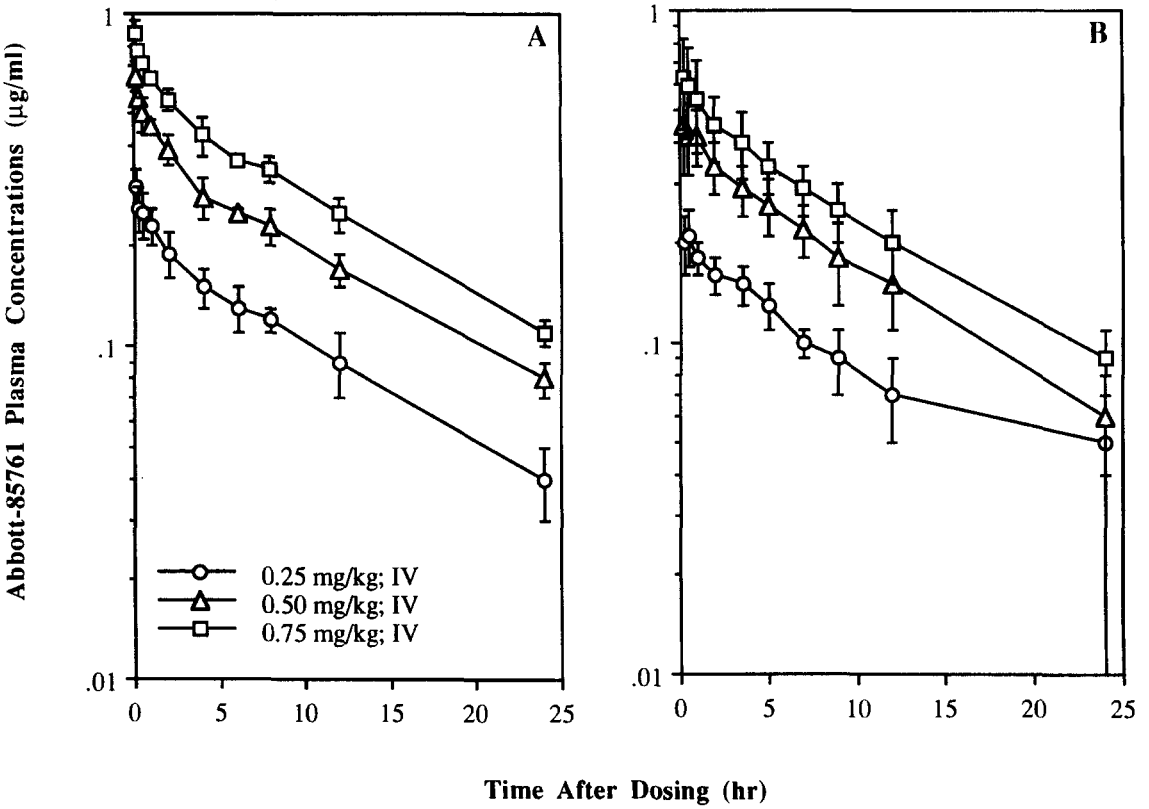


Fig. 2. Mean plasma concentration-time profiles following administration of single doses of Abbott-85761 to beagle dogs. Key: (A) I.V. treatment; bar represent S.D. of three animals. (B) I.T. treatment; bar represent S.D. of six animals. (○) 0.25 mg/kg, (△) 0.50 mg/kg), and (□) 0.75 mg/kg.

0.75 mg/kg of Abbott-85761 in groups of three dogs. Data after I.T. instillation of equivalent single doses of drug in groups of six dogs are also shown in Fig. 2. Estimates of the pharmacokinetic parameters for each of the respective treatments are listed in Table 2. Bioavailabilities of the I.T. doses were estimated using area under the plasma concentration vs. time course curve through 24 h (AUC_{0-24}). The concentrations of Abbott-85761 in plasma declined rapidly after I.V. dosing. The elimination half-life of Abbott-85761 in plasma after I.V. dosing ranged between 9 and 11 h (Table 2). The plasma concentrations of Abbott-85761 increased linearly with increase in drug dose. Hence, the area under the blood drug concentrations vs. time curve (AUC) also increased linearly with increase in the dose of Abbott-85761 ($r^2 = 0.999$).

Following I.T. instillation as a solution, Abbott-85761 concentrations in plasma increased rapidly, with maximum concentrations (C_{max}) attained within 0.3–0.6 h after dosing (Table 2). Considering the extremely low aqueous solubility of Abbott-85761, these results are rather surprising and indicate that the lung might provide for rapid absorption of lipophilic compounds including Abbott-85761. The C_{max} values following I.T. drug administration also increased proportionately with increase in the dose of Abbott-85761. For example, for I.T. doses of 0.25, 0.5 and 0.75 mg/kg, the C_{max} values were estimated to be

Table 2
Pharmacokinetic summary after intravenous and intratracheal dosing of Abbott-85761 to dogs

Abbott-85761 (mg/kg)	T_{max} (h)	C_{max} (h)	$AUC_{0\ 24}$ (μg h/ml)	F (%)	$t_{1/2}$ (h)	MRT (h)
A. Intravenous dosing						
0.25			2.53 ± 0.34		10.86 ± 1.27	15.07 ± 2.20
0.50			4.92 ± 0.49		10.75 ± 0.80	14.86 ± 1.14
0.75			7.13 ± 0.56		9.77 ± 0.84	13.55 ± 1.00
R^2			0.999			
B. Intratracheal dosing						
0.25	0.50 ± 0.27	0.22 ± 0.04	2.11 ± 0.49	0.83	12.85 ± 4.19	18.72 ± 7.34
0.50	0.63 ± 0.41	0.47 ± 0.11	4.06 ± 0.77	0.83	10.67 ± 3.56	14.63 ± 4.72
0.75	0.29 ± 0.10	0.63 ± 0.19	5.59 ± 0.99	0.78	10.66 ± 2.05	14.47 ± 2.80
R^2		0.984	0.995			

0.22 ± 0.04, 0.47 ± 0.11 and 0.63 ± 0.19 μg/ml, respectively. These results suggest that following pulmonary delivery, Abbott-85761 is absorbed in a dose-independent manner.

The absolute bioavailability following I.T. delivery of 0.25, 0.50 and 0.75 mg/kg Abbott-85761 were estimated to be 0.83, 0.83 and 0.78, respectively (Table 2). Like C_{max}, linear dose-AUC proportionality was observed following I.T. treatment, with coefficients of determination $\geq$ 0.995. When the relationship between dose and AUC for individual subjects were examined, no obvious trends of curvature at the high end of the dose range was observed. Based on analysis of variance (ANOVA), formulation type and subjects were found to be the main sources of variation in estimated results for AUC. The effects of sequence and period were insignificant (Table 3).

Fig. 3 displays the correlation between drug AUC following I.V. administration of three doses of Abbott-85761 vs. AUC estimated after I.T. instillation of similar doses of the drug. A good linear correlation was obtained with coefficient of determination ≥ 0.998. The slope of the fitted line, i.e. 0.757, is indicative of pulmonary bioavailability of solution doses of Abbott-85761 within the dose range of 0.25–0.75 mg/kg. It suggests that within the dose range investigated, the pulmonary bioavailability of Abbott-85761 is about 76%. It should be noted that the I.T. instillation of drug, as opposed to I.V. dosing, did not effect the elimination half-life of drug. Regardless

Table 3
Results of analysis of variance (ANOVA) for the sources of variation in AUC

Effect	I.V. dosing (p)	I.T. dosing (p)
Dose	0.009	<0.001
Period	0.210	0.075
Sequence	0.873	0.726
Subject (Sequence)		0.002

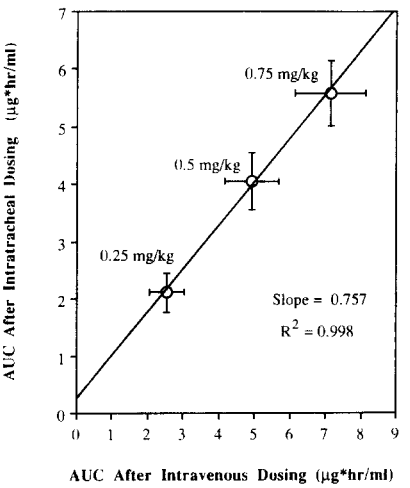


Fig. 3. Correlation between AUC estimated after intravenous administration of three doses of drug vs. AUC estimated after intratracheal instillation of equivalent doses of drug solution to dogs. The bars on x-axis represent S.D. of three animals, and the bars on the y-axis represent S.D. of six animals. A slope of 0.757 indicates that between dose range of 0.25–0.75 mg/kg, the pulmonary bioavailability of instilled drug solution is about 75.7%.

Table 4
Particle size distribution of Abbott-85761 in MDI aerosol formulation

Parameter	Batch 1 (μm)	Batch 2 (μm)	Batch 3 (μm)
Diameter of 10% particles	3.1	2.8	3.2
Diameter of 50% particles	6.8	5.4	7.9
Diameter of 90% particles	19.9	10.5	20.7

Measurements based on volume distribution.

of the drug dose and route of administration, the elimination half-life of Abbott-85761 ranged between 10 and 13 h (Table 2).

The drug plasma concentration data were used to estimate the in vivo mean residence time (MRT) of Abbott-85761 according to the following relationship (Veng-Pedersen, 1989):

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

where AUC and AUMC are the area under the blood concentration [$C(t)$] curve and the area under the first moment curve, respectively; D is the drug dose, and F is the fraction of drug absorbed. AUC as well as AUMC were calculated by trapezoidal approximation. Integration of the data from the last time point (t_n) to infinity was achieved using the relationship

$$\mathrm{AUMC}_{t^*-\infty} = \frac{t_n C(t_n)}{k} + \frac{C(t_n)}{k^2} \quad (2)$$

where k is the terminal rate constant. MRT represents the mean time which drug molecules spend in the body after administration. No significant differences in MRT were observed between I.V. and I.T. treatments at either dose (Table 2). This once again suggests that Abbott-85761 is rapidly absorbed from the lung.

3.2. Pulmonary absorption of aerosolized Abbott-85761

3.2.1. Formulation development

Tetrafluoroethane, HFC-134a, is a gas with a boiling point approximately -26.5°C (Dalby et al., 1990; Gupta and Adjei, 1997b). When subjected to high pressure and low processing temperatures HFC-134a is a very non-polar solvent with extremely poor solubility characteristics (Dalby et al., 1990; Gupta and Adjei, 1997b). Since it is immiscible with most conventional surfactants used in MDI formulations, HFC-134a demonstrates also poor dispersion qualities. For this reason, various excipients were screened to assess their effect on the quality of Abbott-85761 suspensions in HFC-134a. A selected formulation with good dispersion properties (e.g. good homogeneity and no distinct separation for ≥ 1 week) and acceptable chemical stability (i.e. no drug loss at RT over 1 month) was further characterized for functional performance, i.e. particle size distribution, dosimetry and drug content uniformity.

3.2.2. Formulation particle size

Table 4 compares the ex-valve particle size data for three batches of the selected formulation. About 10% of the particles in all three batches of the formulation were ≤ 3.2 μm. Of the particles 50% were ≤ 7.9 μm. This size fraction is slightly larger than the ideal respirable size range of ≤ 5 μm for inhaled pharmaceutical aerosols (Gupta and Hickey, 1991). It was realized that low bioavailability of aerosolized drug may in part reflect less desirable deposition pattern of drug in the peripheral lung due to suboptimal particle size distribution. Nonetheless, in view of the fact that Abbott-85761 particles ≤ 7.9 μm have the potential to deposit in the airways, and Abbott-85761 is completely absorbed following oral administration, the aerosol formulation was considered acceptable for assessment of absorption kinetics and bioavailability of this drug from the lungs.

3.2.3. Shot weight and drug content uniformity

Gravimetric measurements of serially actuated shots of a MDI formulation provide a rapid assessment of shot-to-shot variability. A vial with 50 μl valve should deliver an aliquot of drug formulation equivalent to valve volume multiplied by the density of the formulation. The density of HFC-134a is about 1.22 (Dalby et al., 1990). Hence, a 50 μl valve should deliver about 61 mg/spray or 305 mg/5 sprays. Tentative product specification limits of $\pm$ 10% were assigned to provide a mechanism for evaluating drug dosimetry. Therefore, for a formulation with a 50 μl valve, the acceptable shot weight limits would be 274.5–333.5 mg/5 sprays.

A representative mean shot weight profile over the use-life of an aerosol container containing Abbott-85761 is displayed in Fig. 4. Acceptable dosimetry from this formulation lasted through about spray no. 170, beyond which a sharp 'tail-off' commenced. Including five spray shots for priming the valve, this translates to almost 8.75 ml of formulation deliverable from a container with 10 ml fill volume. The remaining 1.25 ml (or 12.5% v/v) was not deliverable from the device possibly a result of substantial reduction in the internal pressure of the container. This fraction is referred to as dead-volume. Similar shot weight profiles were obtained with other batches of the formulation.

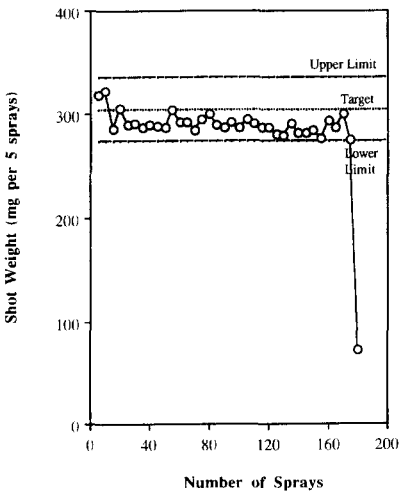


Fig. 4. In vitro dosimetry of Abbott-85761 MDI aerosol formulation. The center line represents target shot weight and window represents $\pm$ 10% limits around the target. Notice sharp 'tail-off' around spray no. 170.

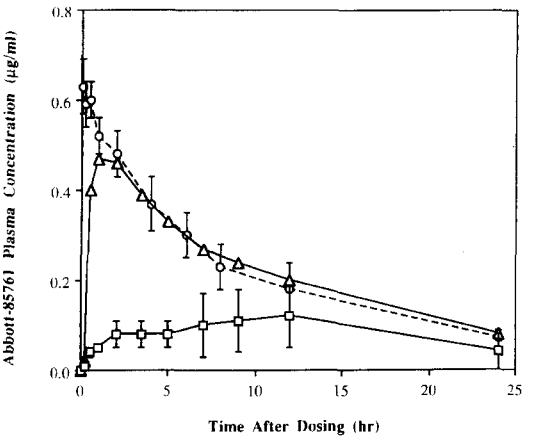


Fig. 5. Mean plasma concentration-time profiles following I.V., oral and inhalation delivery of 0.5 mg/kg of Abbott-85761 to beagle dogs ($n = 9$). Key: (○) I.V. delivery; (△) oral delivery; and (□) aerosol drug delivery. Each point represents the mean $\pm$ S.D. for 9 dogs.

Chemical analysis of drug in emitted spray aids in the estimation of dose uniformity. HPLC analysis of randomly selected sprays from several aerosol containers revealed dose delivery per spray to be well within the limits of $\pm$ 15% of the nominal dose.

3.2.4. In vivo absorption and pharmacokinetics of aerosolized Abbott-85761

In a three-way cross-over study involving nine tracheotomized dogs, the plasma drug concentrations over 24 h following inhalation aerosol administration of 0.5 mg/kg Abbott-85761 were compared with those after oral and I.V. administration of the drug. The results of this study are displayed in Fig. 5. Following oral administration, Abbott-85761 concentrations in the plasma increased rapidly, with maximum concentrations (C_{max}) attained within 1 h after dosing (Table 5). The C_{max} value following oral administration estimated 0.48 ± 0.13 μg/ml and found to be comparable to the highest drug concentration achieved after I.V. drug dosing (i.e. 0.63 ± 0.06 μg/ml). However, drug concentrations after oral and I.V. drug delivery declined rapidly to about

0.2 μg/ml by 12 h after dosing. In contrast, the aerosol formulation demonstrated slow drug absorption which appeared to last for at least 12 h after dosing. Plasma drug concentrations of about 0.1 μg/ml were maintained over this time with the inhalation formulation. The mean T_{max} was estimated to be 6.72 ± 4.10 h (Table 5).

A summary of the bioavailability parameters with the three treatments is presented in Table 5. Based on 90% confidence interval, the oral drug bioavailability was found to be equivalent to the I.V. control. The AUC_{0-24} estimate for the inhalation aerosol treatment was significantly lower than that for oral and I.V. treatments, translating into mean bioavailability of about 40%. This figure is drastically higher than that usually observed with aerosol products and may be attributed to dosing methodology used in this study, i.e. spraying directly into the trachea. Based on ANOVA of AUC_{0-24} data, the route of dose administration ($p < 0.001$) and sequence of dosing ($p = 0.013$) were found to be the main sources of variation. The effects of subject ($p = 0.543$) and period ($p = 0.098$) were insignificant.

Table 5
Pharmacokinetic summary following intravenous, inhalation and oral delivery of 0.5 mg/kg Abbott-85761 to tracheostomized dogs

Parameter	I.V.[a]	Oral[b]	Aerosol[c]
C_{max} (μg/ml)		0.48 ± 0.13	0.14 ± 0.08
T_{max} (h)		1.06 ± 0.39	6.72 ± 4.10
AUC_{0-24} (h*μg/ml)	5.06 ± 1.04	5.09 ± 1.60	1.90 ± 1.05
F (%)	100 ± 0	104 ± 21	41 ± 22
90% confidence interval		86–121	23–60
k (h^{-1})	0.09 ± 0.04	0.09 ± 0.04[b]	0.06 ± 0.02
$t_{1/2}$ (h)	9.27 ± 2.94	9.27 ± 2.94[b]	12.57 ± 5.26
Cl (L/h*kg)	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
V (L/kg)	1.11 ± 0.29	1.35 ± 0.35	1.08 ± 0.35
MRT (h)	7.52 ± 0.81	8.09 ± 0.41	9.48 ± 2.80
MAT (h)		0.81 ± 0.64[a]	3.57 ± 2.24[d]

[a]I.V. dose: 5 ml of 1 mg/ml in 60% v/v polyethylene glycol 400 in water. Only eight animals used in this group.
[b]Oral dose: 20 ml of 0.25 mg/ml in 60% v/v polyethylene glycol 400 in water.
[c]Inhalation dose: 0.5 mg/50 μl spray × 10 sprays/dog; Composition: 10 mg/ml Abbott-85761 in HFC-134a.
[d]$n = 6$.

Table 5 also summarizes some other pharmacokinetic values estimated for oral, I.V. and inhalation delivery of Abbott-85761 in dogs. The elimination half-life ($t_{1/2}$), clearance (Cl), volume of distribution (V) and MRT of drug were comparable for all routes of administration. The mean drug absorption time (MAT) following administration as an inhalation aerosol was estimated according to the following relationship (Veng-Pedersen, 1989):

$$MAT_{Aerosol} = MRT_{Aerosol} - MRT_{I.V.} \quad (3)$$

where $MRT_{I.V.}$ is mean residence time of the drug in the systemic circulation after I.V. administration and $MAT_{Aerosol}$ refers to the mean time involved in the in vivo dissolution of drug and its absorption from the lung. The average $MRT_{I.V.}$ was estimated to be 7.52 ± 0.81 h. The average MRT for the inhalation formulation was estimated to be 9.48 ± 2.80 h. The average MAT for the inhalation formulation, 3.57 ± 2.24 h, was 4-fold higher than that estimated for the orally delivered drug, i.e. 0.81 ± 0.64 h (Table 5). This difference was statistically significant ($p \leq 0.05$).

3.2.5. Absorption analysis of aerosolized Abbott-85761

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

$$C(t) = f(t) * C_d(t) = \int C_d(t - t) f(t)\, dt \quad (4)$$

where $C_d(t)$ is the unit impulse response characteristic of the system. For most pharmacokinetic applications, $C(t)$ and $f(t)$ represent the plasma drug concentration and the rate at which drug enters the system, respectively (Cutler, 1978). $C_d(t)$ is the plasma concentration resulting from the instantaneous input of a unit amount of drug into the system.

In the present study, individual plasma concentration profiles following I.V. administration were used as unit impulse responses $C_d(t)$. The plasma

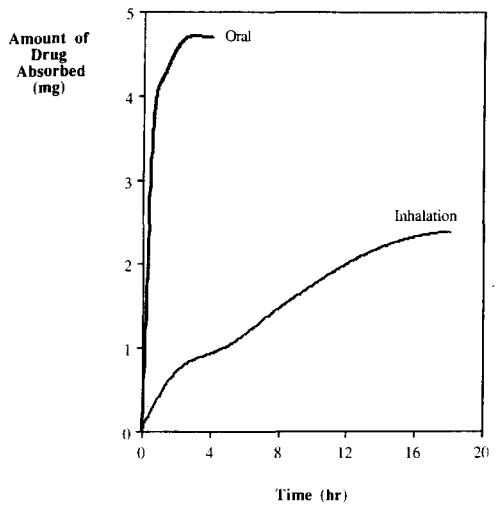


Fig. 6. Mean in vivo absorption of Abbott-85761 estimated by deconvolution after oral and inhalation delivery to dogs ($n = 9$).

drug concentration data from the aerosol formulation or oral solution, $C(t)$, were fitted to a smoothing cubic spline function and then deconvoluted with $C_d(t)$, using the Program PCDCON (University of Texas, Austin, Texas), to obtain apparent in vivo drug absorption profiles. The results demonstrated slow and prolonged absorption of Abbott-85761 with the MDI formulation which lasted for ≥ 16 h. However, oral drug absorption was essentially complete within 2–3 h of dosing (Fig. 6).

The 'pulmonary bioavailability' of Abbott-85761 from the aerosolized formulation was estimated as the plateau value using the apparent in vivo absorption profiles obtained by deconvolution (Cutler, 1978) and found to be approximately 46.7%. These results are comparable to the relative bioavailability of the formulation estimated from the AUC data (i.e. $41 \pm 21\%$; Table 5).

3.2.6. Comparative performance of aerosolized and instilled Abbott-85761

If the in vivo distribution data observed with aerosolized Abbott-85761 at a dose of 0.5 mg/kg is compared with an equivalent dose of instilled drug, a significant effect of formulation and dosing technique becomes instantly apparent (Fig. 7). Firstly, the solution formulation instilled into the trachea was absorbed significantly faster than drug delivered from the MDI formulation. This is reflected in T_{max} value of 0.63 ± 0.41 h for the instilled drug vs. 6.72 ± 4.1 h for the aerosolized drug. This difference is rather too large than that theoretically anticipated on the basis of formulation composition and dosing technique, and may be explained by either of the following factors:

(i) the presence of cosolvents (i.e. 50% v/v PEG 400 and 25% v/v ethanol in water) causing transient changes in the pulmonary epithelia and allowing for faster drug absorption,

(ii) the presence of cosolvents minimizing precipitation of drug in vivo. It is probable that the cosolvents maintain the drug in solution for prolonged periods during which the absorption is essentially complete, and

(iii) particle size of drug, rather than the site of deposition, is critical in drug absorption. Indeed the drug is well absorbed after oral administration. Hence, it may not require lower airway deposition for efficient absorption. Despite the fact that instillation techniques deliver drug centrally in the lung, Abbott-85761 appears to be well absorbed.

Further experiments investigating the plasma distribution of drug following I.T. instillation as a suspension of increasing particle size may be required to confirm the above two hypothesis.

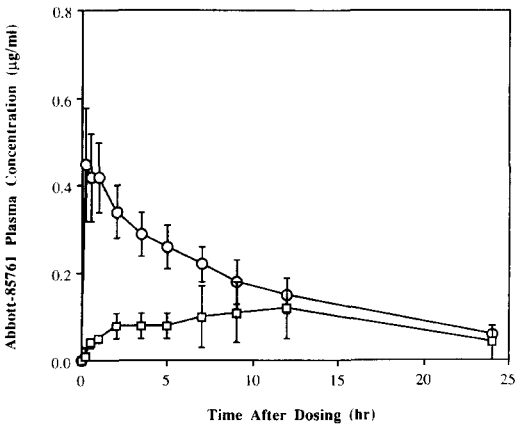


Fig. 7. Mean plasma concentration-time profiles following intratracheal instillation (○) and inhalation aerosol delivery of 0.5 mg/kg of Abbott-85761 to beagle dogs. Each points represents the mean ± S.D. for 6–9 dogs.

The second key observation of I.T. vs. inhalation aerosol treatment is significant difference in AUC's (Fig. 7). The 41% bioavailability for the aerosolized drug is significantly lower than 83% bioavailability estimated for the drug instilled as a solution (Tables 2 and 5). These results are contradictory from earlier published reports indicating higher bioavailability with inhalation delivery vs. I.T. instillation (Colthrope et al., 1993; Farr and Taylor, 1997; Niwen, 1997). This may be attributed to one or both of the following factors:

(i) use of different animal models (i.e. isolated lung perfusion models, rabbits, and monkeys vs. dogs in the current study) and different dosing techniques (i.e. nebulization vs. I.T. aerosolization in the current study);

(ii) loss of drug via the exhaled air. This was observed immediately after the administration of each spray into the trachea of dogs;

(iii) formation of thick mucus plug near the site of drug delivery in the trachea preventing diffusion of drug to the epithelial surface of the membrane. This was also observed after aerosolization of drug to the dogs. Several 5-lipoxygenase inhibitor compounds investigated in our laboratory have demonstrated good oral and pulmonary permeability (Data on file, Abbott Laboratories); however, they exhibit dissolution controlled absorption (Qiu et al., 1995; Data on file, Abbott Laboratories). In the current study, it seems that poor dissolution of water-insoluble drug from the thick mucus plug may have hampered quantitative absorption of the aerosolized drug.

4. Conclusions

Results from this study indicated that despite a limited solubility, an aqueous formulation of Abbott-85761 is rapidly absorbed from the lung of dogs. For the three dose levels investigated following I.T. administration, i.e. 0.25, 0.50 and 0.75 mg/kg, complete absorption of Abbott-85761 occurred within 15–30 min after dosing. Plasma concentrations of drug increased in proportion to the dose of drug, yielding linear dose–AUC response ($R^2 = 995$). The absolute pulmonary bioavailability in the dose range of 0.25–0.75 mg/kg, against I.V. controls, was estimated to be about 76%.

A HFC-134a based MDI aerosol formulation of Abbott-85761 demonstrated reproducible dose delivery in vitro. In dogs, the inhalation formulation demonstrated prolonged absorption of drug from the lung and resulted in peak plasma drug concentrations which were about one-fourth of those observed following I.V. and oral drug administration. The absolute pulmonary bioavailability of aerosolized drug approximated 41%. The data also indicate significant effect of formulation composition (i.e. aqueous solution vs. suspension MDI) and dosing technique (i.e. I.T. instillation vs. I.T. aerosolization) on in vivo distribution of equivalent doses of drug. Instilled solutions yielded faster and quantitative absorption of drug than aerosolized formulations. Aerosolization of Abbott-85761 resulted in slow drug absorption and bioavailability was approximately 50% of that estimated after instillation of an aqueous solution of the drug.

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