The Pharmacokinetics of Pulmonary-Delivered Insulin: A Comparison of Intratracheal and Aerosol Administration to the Rabbit

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The pulmonary deposition and pharmacokinetics of insulin, administered via an endotracheal tube as an aerosol and instillate, in formulations containing either ^{113m}In-DTPA or ^{99m}Tc-DTPA (for gamma scintigraphic imaging) have been studied in four male New Zealand White rabbits. Using a randomized crossover design, the pharmacokinetics of intravenous insulin were also characterized. Recovery of immunoreactive insulin after nebulization was greater than 90%, indicating that the aerosolisation procedure did not cause appreciable insulin degradation. Gamma scintigraphy demonstrated that the penetration index (peripheral:central deposition) for the aerosolized formulation (1.52) was much greater than that for the instillate (0.32). Gamma scintigraphy also allowed exact quantification of the dose deposited after aerosol administration and thus permitted accurate determination of bioavailabilities. The bioavailable fraction for aerosolized insulin was 10-fold greater than for instilled insulin (57.2 vs 5.6%). Mucociliary clearance was likely to be greater for the instillate since it showed a preferential central deposition; this may account for the lower bioavailability. Insulin pharmacokinetics from both pulmonary formulations were absorption rate limited, resulting in postpeak half-lives which were approximately 20-fold greater than the intravenous elimination half-life (3 min). The apparent absorption rate constants resulting from instillation and aerosolisation were statistically equivalent (0.015 and 0.011 min^{-1}, respectively). Mucociliary clearance of insulin would result in an overestimation of the true absorption rate constant; hence if mucociliary transport were greater for the instillate, then the true airways to blood transfer rate constant will be higher for the aerosolized formulation.

KEY WORDS: insulin; aerosol; pulmonary; pharmacokinetics; gamma scintigraphy; drug delivery; rabbit.

INTRODUCTION

Many future therapeutic agents are likely to be proteins or polypeptides. Enzymatic degradation of such agents restricts the gastrointestinal tract as a route of absorption to the systemic circulation; therefore, alternative methods of delivery need to be addressed. A large surface area, extensive vasculature, thin permeable membrane, and relatively low extracellular enzymic activity constitute a theoretically favorable absorptive environment for proteins and polypeptides following aerosol delivery. Airway-to-blood transport of high molecular weight compounds may depend upon the site of deposition and surface area available for diffusion, which, in turn, are a function of the method of solute delivery.

Intratracheal (i.t.) instillate administration provides a rapid quantifiable method of delivery but distribution tends to be localized and uneven (1); thus, absorption results from deposition on only a small fraction of the total surface area of the lung. Administration by aerosol results in a more uniform distribution with greater penetration into the alveolar region (1,2); however, it is difficult to determine exact doses deposited. Brown and Schanker (3) studied 12 compounds of varying lipid solubility ranging in molecular weight from 59 to 734. Using a rat model, they demonstrated that aerosol administration resulted in absorption rates twofold greater than those seen after i.t. delivery. Similar findings were also reported from studies in the mouse and rabbit (4). The differences in absorption rates were attributed to disparity in mucosal permeability.

In this paper the pharmacokinetics of insulin (MW 5700), following i.t. instillation and aerosol administration in a rabbit model, were investigated. Aerosols were generated from aqueous solutions by air-jet nebulization and characterized using a light-scattering method and a standard inertial separation technique. The extent and site of solute deposition in the lung were monitored by gamma scintigraphy to assist in the interpretation of pharmacokinetic findings and to calculate exact insulin doses administered by aerosol.

MATERIALS AND METHODS

Aerosolization of Insulin

A primary consideration in the design of the system used for aerosol administration of insulin was to exclude the possibility of solute deposition in the oropharyngeal region. A system was designed, therefore, in which an aerosol cloud was generated into a holding chamber, then transiently held before inhalation by anesthetized rabbits via an endotracheal tube. Insulin solutions (3 ml, 300 U ml^{-1}) were prepared by dissolving highly purified crystalline bovine insulin (24.4 U mg^{-1}; Sigma Chemical Co., UK) in 0.85% NaCl and adjusting to pH 4 with 0.1 *M* HCl. Aerosols were generated from these solutions using an air-jet nebulizer (Turret, Medic-Aid Ltd., UK) operating at 10 l min^{-1} for 24 sec and directed into the 4-L high-grade polyethylene holding chamber. The total amount of insulin delivered to the holding chamber was estimated from the nebulizer weight loss during the aerosolization period. Separate studies established that the insulin concentration in the nebulizer solution did not change during this short period.

Mass median diameter (MMD) of the aerosol as a function of holding time (1–6 min) was determined with a laser diffraction particle sizer (Model 2600, Malvern Instruments, UK) following expulsion of the chamber contents into the laser beam. Aerosol characterization was also carried out by connecting the holding chamber to a previously calibrated multistage liquid impinger (MLI) operating at 60 l min^{-1}. Total airborne insulin within the bag was calculated by UV spectrophotometry (276 nm) of the aerosol collected on the

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throat, stages, and terminal filter of the MLI. In addition, mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) were derived from insulin deposition on each stage, and the respirable fraction (RF; i.e., droplets <5.5 μm) was estimated from the sum of the amount of insulin on stages 3 and 4 and the terminal filter. In duplicate experiments insulin stability to the nebulization process was determined by the addition of 5 MBq ml^{-1} ^{113m}In-DTPA to the insulin solutions prior to aerosol generation. The radiochemical was prepared by an established method (5) in which DTPA (Sigma Chemical Co., UK) is complexed with $^{113m}InCl_3$ eluted from a sterile generator (Amersham International, UK). Following nebulization directly into the MLI, the deposition of insulin and the radionuclide at each location was determined by RIA and gamma counting, respectively. In addition to a total mass balance, any discrepancy in the ratio of insulin concentration:^{113m}In activity between the original solution and the various sites of deposition was taken as a measure of insulin degradation upon nebulization.

Pharmacokinetic Studies

A crossover study of i.v., i.t., and aerosol administration was conducted in four male New Zealand White rabbits (4.1 ± 0.4 kg) with a minimum washout period of 7 days. After an overnight fast to reduce basal insulin levels, the rabbits were transiently anesthetized with a combination of Hypnorm (Janssen Animal Health, UK), 0.1 ml kg^{-1} i.m., and Hypnovel (Roche Laboratories, UK), 0.2 mg kg^{-1} i.v., before receiving bovine insulin as a pH 4 isotonic solution. Insulin, 0.1 U kg^{-1}, was given via a marginal ear vein in the i.v. studies. For i.t. and aerosol delivery, the anesthetized animal was intubated with a polyethylene, endotracheal tube (i.d., 3 mm; o.d., 4 mm; Portex, UK) coated with 2% xylocaine gel (Astra Pharmaceuticals, UK). The i.t. instillates of 5 U kg^{-1} of insulin (in approximately 1 ml of solution) were administered slowly through a polyethylene dosing tube (0.4-mm i.d., 0.8-mm o.d.; Portex, UK) inserted into the endotracheal tube such that its tip was at the bifurcation of the trachea. The tubing was then washed out with aliquots of air and removed together with the endotracheal tube. For aerosol administration, a solution of 300 U ml^{-1} insulin was nebulized into the holding chamber. After 2 min, the endotracheal tube was connected and the rabbit allowed to breathe aerosol for a further 4 min, before the tube was removed. Venous blood samples (0.6 ml) were collected from a marginal ear vein via a heparinized (300 U ml^{-1}) indwelling catheter at 0, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, and 180 min after dosing. Additional samples were taken at 15 and 25 min following i.v. dosing and sampling was terminated at 60 min. Samples were collected into chilled, heparinized tubes and centrifuged immediately at 3000 rpm. Plasma was then stored at −20°C before analysis in duplicate by radioimmunoassay (RIA) (Amersham International, UK).

The pharmacokinetic parameters, clearance (CL), steady-state volume of distribution (V_{ss}), and bioavailable fraction (F) were calculated from the area under the plasma concentration–time (AUC) and area under the plasma concentration * time–time (AUMC) profiles using standard methodology (6). Logarithmic trapezoidal summation was used in AUC calculations during periods of declining plasma concentrations, while linear trapezoidal summation was used at other times and for AUMC calculations. Terminal rate constants, used for extrapolation to infinity, were determined using a nonlinear least-squares regression program, Minim. Using weighting factors of $1/y$ or $1/y^2$, the complete intravenous profiles and the postpeak concentrations following i.t. and aerosol dosing were all accurately characterized by single-exponential functions. Goodness of fit was assessed by residuals analysis. The intravenous elimination rate constant (k) was much greater than the terminal rate constants seen after i.t. and aerosol dosing; hence the latter are denoted absorption rate constants (k_a).

Gamma Scintigraphic Studies

The purpose of undertaking gamma scintigraphy was to quantify the amount and the site of solute deposition within the lung following aerosol and instillate delivery. Posterior and anterior lung perfusion images (120 s) were recorded on a gamma camera (Maxicamera 400A, General Electric, UK), fitted with a medium-energy parallel-hole collimator, following the i.v. administration of 4 MBq ^{99m}Tc-labeled macroaggregated albumin (Medical Physics Department, University Hospital of Wales, UK). An energy window setting of ±10% maximum keV was used together with an image magnification factor of 2.0. A region of interest depicting the 15% contour was constructed around each side of the lung and stored for use as a lung template in later studies. Employing a similar scintigraphic procedure, the pattern of solute deposition in the lung was visualized following the administration of solutions containing 2 MBq ^{113m}In-DTPA or 4 MBq ^{99m}Tc-DTPA (Medical Physics Department, University Hospital of Wales, UK). The appropriate lung perfusion template was superimposed and a horizontal profile (64 pixels in length) transecting the perihilar region drawn. Regional distribution in the right lung was quantified by division of the profile length between the lung margins into 2/5 and 3/5 sections. The total count (minus background) within each region was calculated to yield the proportion of activity in the central and peripheral zones of the lung, respectively. Total (left and right) lung count following aerosolized delivery was also determined and expressed as an absolute activity following appropriate correction for attenuation. This was calculated by comparison of corrected counts derived from images following the i.t. administration of 0.5, 1, 2, and 5 MBq of the radiopharmaceutical in 1 ml of 0.9% saline in a separate animal with the counts obtained for the same amount of activity dispersed in a volume of saline contained in a perspex box of similar dimension to rabbit lungs. In the aerosolized insulin experiments, absolute counts were related to amount of insulin deposited by similarly counting 0.1 ml of nebulizer solution in the phantom lung and using the previously determined attenuation factor.

RESULTS AND DISCUSSION

In contrast to particulate aerosols, the size distribution of droplets generated from aqueous solutions in a nebulizer will alter with time as a result of solvent evaporation. As the method employed for aerosol delivery to rabbits required that the nebulized droplets were transiently held prior to

administration, it was important to restrict such changes to a minimum over the administration period. As demonstrated in Table I, there was a dramatic reduction of >45% in the amount of airborne insulin within the holding chamber 1–2 min postnebulization, which was followed by a further, smaller reduction of around 20% after 6 min. It is likely that the initial reduction in insulin concentration resulted from a combination of inertial and electrostatic precipitation of the aerosol on the walls of the polyethylene chamber. During the holding period, MMD (and MMAD) gradually reduced, probably due to solvent evaporation, with the effect that the amount of insulin in the RF remained relatively constant between 2 and 6 min. Consequently, a protocol of a 2-min holding period before a 4-min administration period was adopted in these studies. It should be noted, however, that the RFs, measured with the MLI, are likely to be overestimated owing to the significant evaporation of the aqueous aerosol droplets during the sampling procedure (7). While it has been stated (8) that aerosolization of aqueous solutions by nebulizer can denature proteins, the molecular integrity of insulin during the short nebulization procedure in this study was demonstrated (Fig. 1). The concordance in insulin concentration:^{113m}In-DTPA ratios between the original solution and all stages of the MLI demonstrates a lack of insulin degradation. Furthermore, mass balance indicated 92.53 ± 1.45% (mean ± SD) recovery of the nebulized insulin, comparable to a recovery of 88.20 ± 5.50% for the radiolabeled complex.

Critical differences in the deposition pattern were obtained following the two modes of pulmonary administration (Fig. 2), where a preferential central deposition was evident following i.t. administration. This is further demonstrated by the significant ($P < 0.025$, paired t test) difference in mean penetration index (peripheral:central counts) for i.t. (0.32 ± 0.08) and aerosol (1.52 ± 0.36) delivery. As the rate of mucociliary transport is known to increase progressively from the peripheral to the central regions of the respiratory tract (9), the rate of clearance of a slowly absorbed solute will be influenced by its site of deposition. In these studies, therefore, it is likely that the extent of insulin removal by mucociliary clearance was greater following i.t. than aerosol delivery, thus providing a competitive process to absorption. This hypothesis is supported by the markedly lower bioavailable fraction (F) for i.t. instillation (2.9–10%) compared with

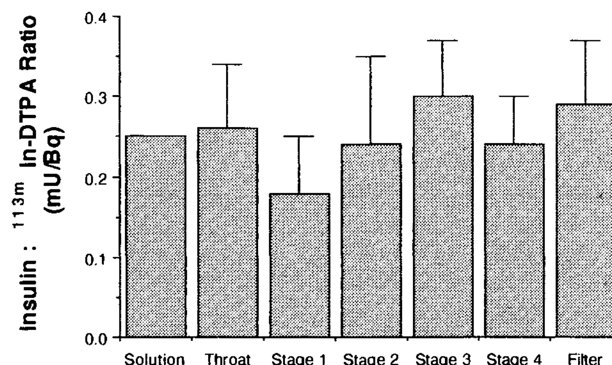

Fig. 1. Comparison of the insulin:^{113m}In-DTPA ratio at each location of the MLI with that of the original nebulizer solution (mean ± SD, n = 3).

that following aerosol dosing (30–91%), as reported in Table II. The bioavailable fraction for i.t. instilled insulin in this study is much lower than that previously reported in the rat (10). The disparity may be species related; however, it is noteworthy that in the rat study, a tracheostomy tube was used, which inhibited mucociliary clearance.

A significant advantage of combining gamma scintigraphic and pharmacokinetic methodologies is the ability to determine accurately the extent of solute deposition from an aerosol in the lung, from which an absolute bioavailable fraction may be calculated with reference to i.v. pharmacokinetic data. In contrast to studies involving direct instillation, previous reports of solute bioavailability following aerosolized administration are scarce. One obvious reason for this is that drugs administered to the lung are largely intended for local activity, for which an estimation of bioavailability may not be essential or appropriate. The lung, however, is increasingly being considered as a portal for systemically acting drugs, particularly peptides and proteins, demonstrating a tenable need for accurate determination of bioavailability. Adjei and Garren (11) presented pulmonary absorption data for leuprolide acetate delivered to volunteers from various, pressurized metered-dose inhalers. Bioavailabilities calculated from the dose emitted by the inhalation device were substantially smaller than those estimated from the RF (% <4.7 μm), determined by two *in vitro* methods. While consideration of the RF does acknowledge the

Table I. The Influence of Holding Time on Size Distribution of an Insulin Aerosol Generated from a Turret Nebulizer Operated at 10 L min^{-1}

Holding time (min)	Total airborne insulin (U ± SD)[a]	MMD (μm)[b]	MMAD ± σ_g (μm)[c]	Respirable fraction (%)	Insulin in respirable fraction (U ± SD)
1	47.8 ± 6.8	3.42	2.73 ± 1.98	77.0	36.8 ± 5.7
2	39.2 ± 4.5	3.29	2.63 ± 1.86	79.6	31.2 ± 3.8
4	33.5 ± 6.5	2.80	2.39 ± 1.91	89.3	29.9 ± 6.5
6	31.2 ± 4.1	2.40	1.91 ± 1.97	88.1	27.5 ± 3.0

[a] Total insulin recovered within the MLI. Amount of insulin nebulized into the holding chamber was 72 U, estimated from the difference in weight of the nebulizer unit before and after nebulization.
[b] Mass median diameter determined with Malvern laser diffraction sizer.
[c] Mass median aerodynamic diameter determined with the MLI.

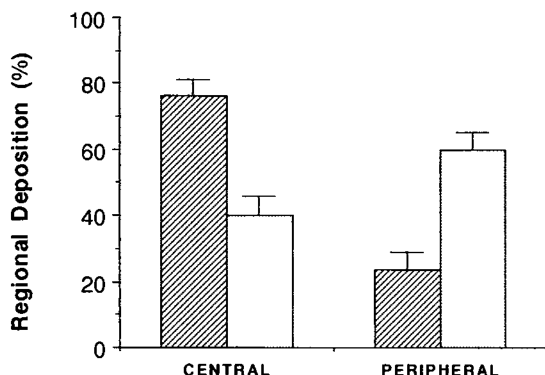

Fig. 2. Regional distribution of activity in the central and peripheral zones across a horizontal profile of the right side of the lung following i.t. (hatched bars) and aerosol administration (open bars), as determined by gamma scintigraphy (mean ± SD, n = 4).

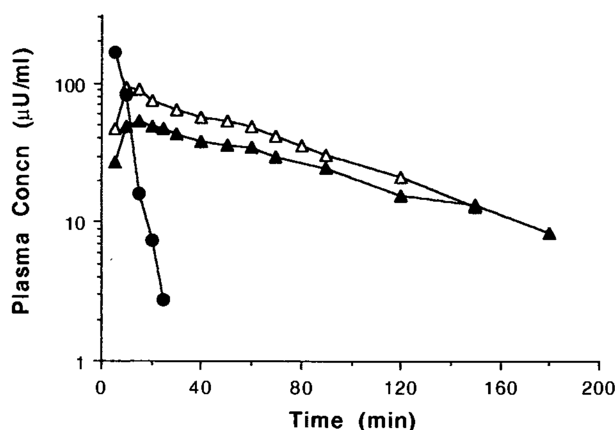

Fig. 3. Mean (n = 4) plasma concentration profiles of insulin following i.v. (●), i.t. (△), and aerosol (▲) administration. Refer to Table II for statistical treatment of data.

importance of particle size in relation to deposition, the actual dose to lung cannot be derived from this parameter. There are substantial correlation data to suggest that an *in vitro* derived RF of a therapeutic aerosol significantly overpredicts the proportion of aerosol capable of penetration and deposition within the respiratory tree, particularly with respect to the alveolar region (e.g., Ref. 12). Byron (8) has suggested a method for estimating the dose deposited in lung, based on a knowledge of aerosol concentration and size distribution, time of administration, and appropriate pulmonary function data. It is instructive to consider, therefore, whether the actual dose to lung in these experiments could be similarly determined. Based on a minute volume of 0.62 L for rabbits (13), a 4-min administration period in our studies should have yielded an insulin dose >12 U, even after accounting the steady decline in airborne insulin concentration within the chamber over this time period. In practice, reproducibly, lower doses of <2 U (1.78 ± 0.06, n = 4) were achieved, a probable consequence of aerosol impaction within the relatively narrow confines of the endotracheal tube and the lack of mixing between inspired and expired air within its small dead volume; no correction could be applied to these factors. Another potential source of error is the use of a literaure value for minute volume. This may be greater than that achieved by rabbits in this study, which were held under light anesthesia during the administration period.

Insulin plasma profiles following i.v., instillate, and aerosol delivery are shown in Fig. 3. Pharmacokinetic parameters derived from these data are given in Table II.

Table II. Insulin Pharmacokinetic Parameters (Mean ± SD, n = 4)

	i.v.	i.t. instillate	Aerosol
CL (ml min^{-1} kg^{-1})	39.34 ± 9.18	—	—
V_{ss} (ml kg^{-1})	196 ± 53	—	—
F (%)^a	100	5.6 ± 3.3*	57.2 ± 28.5*
k (min^{-1})	0.223 ± 0.027		
k_a (min^{-1})	—	0.015 ± 0.004	0.011 ± 0.006
t_{max} (min)	—	11.3 ± 4.8	12.5 ± 2.9

^{a} F values are calculated relative to 100% after i.v. dosing.
* Significantly different (P < 0.05) using paired t test.

Plasma concentrations after i.v. administration declined monoexponentially with a half-life of 3 min. This short half-life resulted from an intermediate clearance and a volume of distribution which approximates that of extracellular fluid. These parameters are proportionate to those of insulin in nondiabetic humans (14). Following both aerosol and i.t. administration peak insulin concentrations were achieved within 15 min. The t_{max} values were not significantly different for the two formulations and are similar to those reported in a previous study (15) involving the pulmonary delivery of insulin via a pressurised inhalation aerosol to rabbits. Postpeak half-lives were approximately 20-fold longer after aerosol (69 min) and i.t. (49 min) dosing than for i.v. dosing (3 min), indicating that absorption rate-limited kinetics prevailed; consequently postpeak half-life was used for calculating absorption rate constant (k_a). Values for k_a were statistically equivalent following aerosol and i.t. administration; this suggests that the rate of insulin absorption is independent of the mode of pulmonary administration. In previous studies, using a range of solutes, Brown and Schanker (3) and Schanker *et al.* (4) reported absorption rate constants following aerosol administration which were roughly twofold greater than those seen after i.t. dosing. In those studies, however, mucociliary clearance was inhibited throughout the experiment by a tight-fitting tracheal cannula. As discussed previously, it seems likely that in our studies mucociliary clearance had a much greater influence on the fate of the i.t. than on the aerosolized insulin. A competitive process to absorption is well known to result in an overestimation of the true absorption rate constant (16). Therefore the equivalent values of k_a observed after i.t. and aerosol administration suggest that the true airway-to-blood transfer rate constant following i.t. delivery is likely to be lower than that following aerosol administration.

In summary we have demonstrated that insulin is efficiently absorbed from the rabbit lung after aerosol administration. The studies have also highlighted major differences in extent of absorption between i.t. instilled and aerosolized insulin, which seems likely to result from differential effects of mucociliary clearance. Gamma scintigraphy has proved a vital technique in comparing insulin pharmacokinetics fol-

lowing i.t. and aerosol administration. It provides a unique opportunity to determine the precise site and extent of solute deposition within the lung, information which has highlighted the potential effects of a competitive process such as mucociliary clearance to the absorption of a slowly absorbed solute from the lung. Appropriate correction of absorption rate constants for mucociliary transport, which can also be derived by gamma scintigraphy, should result in the derivation of true airway-to-blood transfer constants and a valid assessment of the permeability of the lung epithelium to higher molecular weight compounds such as peptides and proteins.

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