The Influence of Regional Deposition on the Pharmacokinetics of Pulmonary-Delivered Human Growth Hormone in Rabbits

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The pulmonary deposition and pharmacokinetics of human growth hormone (hGH), administered by aerosol and instillate, in formulations containing ^{99m}Tc-DTPA (for gamma scintigraphic imaging) have been studied in five male New Zealand White rabbits. Gamma scintigraphy indicated that the peripheral:central deposition tended to be greater for aerosol (1.54) than for instillate (0.8). Two gamma scintigraphic methods were used to quantify dose deposited by aerosol, which permitted bioavailabilities to be determined. The bioavailable fraction for aerosolized hGH (45%) was greater than for instilled hGH (16%). This was attributed to the differential effects of mucociliary clearance. Absorption rate limited pharmacokinetics prevailed for both hGH formulations with post-peak half-lives approximately 10-fold greater than the intravenous elimination half-life of 40 min. Apparent absorption rate constants resulting from instillation and aerosolization were equivalent (0.0012 min^{-1} and 0.0020 min^{-1} respectively), however lung-to-blood transfer rate constants for aerosol delivery (0.00071 min^{-1}) were greater than for instillation (0.00018 min^{-1}).

KEY WORDS: growth hormone; pulmonary; pharmacokinetics; gamma scintigraphy; drug delivery; rabbit.

INTRODUCTION

Human growth hormone (hGH) is currently administered to growth hormone deficient children as daily, or alternate-day, subcutaneous or intramuscular injections, over a number of years. Chronic administration of drugs via transmucosal routes is more acceptable to patients than more invasive parenteral routes. In the absence of penetration enhancers and/or bioadhesive systems the nasal bioavailability of hGH (MW 22,000) in rats is very poor (<1%) (1). Enhancers may cause toxicity upon chronic administration, therefore the possibility of delivering hGH by the pulmonary route has been investigated. Patton *et al.* (2) estimated a bioequivalence of 40% (relative to subcutaneous dosing) and an absolute bioavailability of 10%, following aerosolized hGH administration to the lungs of hypophysectomised rats.

In earlier studies (3,4) we demonstrated the effect of site of deposition upon airways-to-blood transport of insulin (MW 5700) and oxytocin (MW 1007) respectively. Absorption rate constants (k_a) were equivalent following delivery by intratracheal (i.t.) instillation, and by aerosol. This finding was in contrast to those of previous studies with other compounds (5,6) in which absorption was slower following i.t. delivery. We attributed the disparity to the differential effects of mucociliary clearance which was likely to have a greater influence on the instillate in our studies.

In this report the pharmacokinetics of hGH following i.t. instillation and aerosol administration in the rabbit were investigated. Using a simple pharmacokinetic model, absorption rate constants were resolved into components for lung-to-blood transfer (k_{lb}) and elimination from the lung (k_{le}). A radiolabelled complex (^{99m}Tc-DTPA) was included in formulations for pulmonary administration, which permitted the extent and site of solute deposition in the lung to be monitored by gamma scintigraphy.

MATERIALS AND METHODS

A crossover study of intravenous (i.v.), intratracheal (i.t.), and aerosol administration was conducted in five male New Zealand White rabbits (3.8 ± 0.4 kg) with a washout period of 7 days. The animals were transiently anaesthetised prior to dosing with a combination of fentanyl citrate (Sigma Chemicals, Gillingham, UK) 0.026 mg kg^{-1} i.m., droperidol (Sigma) 0.28 mg kg^{-1} i.m., and Hypnovel (Roche Laboratories, Welwyn, UK) 0.2 mg kg^{-1} i.v. In i.v. studies 100 μg kg^{-1} hGH was given via a marginal ear vein. For i.t. and aerosol delivery the anaesthetised animal was intubated as described previously (3). The i.t. instillates contained 2 mg kg^{-1} hGH with 1 MBq kg^{-1} ^{99m}Tc-DTPA (Medical Physics Department, University Hospital of Wales, UK), in 1 mL of modified Sørensen's phosphate buffer (0.067 M; pH 7.4) rendered isotonic with NaCl, and were delivered at the bifurcation of the trachea (3). Solutions of hGH with ^{99m}Tc-DTPA (20 mg mL^{-1}; 10 MBq mL^{-1}) in buffer were aerosolized for 24 sec into a holding chamber, using an air-jet nebulizer (3). Separate studies established that the hGH concentration within the nebulizer did not change during this short period. Following a holding time of 2 min to optimise the concentration of respirable droplets (<5.5 μm diameter) the aerosol was inhaled by anaesthetised rabbits via an endotracheal tube for a period of 4 min. An identical chamber which had been filled with aerosol and held for 2 min was then immediately connected to the endotracheal tube to produce a total exposure period of 8 min, after which the tube was removed from the trachea.

Following nebulization directly into a previously calibrated multistage liquid impinger the deposition of hGH and radionuclide at each location was determined by radioimmunoassay (RIA) and gamma counting respectively. A consistent ratio of hGH concentration: ^{99m}Tc activity in the original solution and the various sites of deposition was taken to indicate that ^{99m}Tc-DTPA was a suitable marker of lung deposition for hGH. In addition, the nebulizer unit was weighed before and after the nebulization period. The concordance in mass balance between hGH and radionuclide was taken as further evidence that hGH degradation did not occur during nebulization.

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Venous blood samples (0.9 mL) were collected from a marginal ear vein via a heparinized (300 U mL^{-1} indwelling catheter at 0, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 1440, and 1445 min after pulmonary dosing. A mean plasma concentration was calculated from the last two time points. Blood was sampled at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, and 180 min after i.v. dosing. The samples were collected into chilled, heparinized tubes and centrifuged immediately at 1800 g. Plasma was separated and then stored at −20°C before analysis, in duplicate, by RIA (ICN Biomedicals Inc., USA). The intra-day coefficient of variation for the assay of hGH at 15 ng mL^{-1} was found to be 5.9%. 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 (7). Terminal rate constants, used for extrapolation to infinity, were determined using a nonlinear least-squares regression program, Minim (R D Purves, Department of Pharmacology, University of Otago, New Zealand). Individual i.v. profiles were characterised by biexponential functions and a first-order absorption, one-compartment disposition model was fitted to the pulmonary data using weighting factors of 1/y and $1/y^2$. Goodness-of-fit was assessed by residuals analysis and was used to determine which weighting factor gave the superior fit. The i.v. elimination rate constant was much greater than the post-peak rate constant seen after pulmonary dosing. The latter is therefore denoted as absorption rate constant (k_a).

The addition of a radiolabelled complex to solutions for pulmonary delivery allowed the amount and site of solute deposition in the lung to be quantified following administration. Anterior and posterior solute deposition images (120 s) were recorded using gamma scintigraphic procedures described previously (3), except that a high resolution low-energy parallel-hole collimator was used in the current studies. A penetration index, was calculated from the ratio of counts in peripheral to central regions of the right lung (3). Total (left and right) lung count (geometric mean of anterior and posterior) following aerosolized delivery was determined and expressed as an absolute activity following appropriate correction for attenuation, which was calculated by two methods. In the first method, corrected counts derived from images following the i.t. administration of 0.5, 1, 2, and 5 MBq ^{99m}Tc-DTPA in 1 mL buffer in a separate animal were compared with the counts obtained for the same amount of activity dispersed in a volume of buffer contained in a perspex box of similar dimensions to rabbit lungs. The second method involved measuring transmittance of activity from a ^{99m}Tc flood source through each animal, and comparison with a calibration curve of transmittance through perspex shields (1–20 cm) to yield an equivalent perspex thickness. The reciprocal of transmittance through half this thickness of perspex gave a value for attenuation of activity by those tissues surrounding the rabbit lung. In the aerosolized hGH experiments absolute counts were related to amount of hGH deposited by counting 0.1 mL of nebulizer solution and using the attenuation values determined in the two methods as described above.

RESULTS AND DISCUSSION

Fig. 1 shows ratios of hGH concentration to ^{99m}Tc-DTPA activity in the nebulizer solution and on each stage of the MLI following nebulization. These ratios were statistically equivalent (one-way analysis of variance; $p > 0.05$), therefore ^{99m}Tc-DTPA could be used with confidence as a marker of hGH deposition. While it has been reported that aerosolization of aqueous solutions by nebulizer can denature proteins (8) there was no significant difference (paired t-test) between total recoverable percentages of hGH (86.71 ± 3.47) and ^{99m}Tc-DTPA (84.20 ± 6.37) calculated by mass balance. This suggests that the molecular integrity of hGH was maintained during the short nebulization procedure employed in this study.

Differences in deposition patterns following the two modes of pulmonary delivery were quantified by means of a penetration index (PI) calculated from the ratio of peripheral:central counts. Mean PI tended to be lower ($p = 0.07$, paired t-test) for i.t. delivery (0.80 ± 0.32) than for aerosol administration (1.54 ± 0.50), indicating a more central deposition of the instillate. Deposition patterns following aerosol delivery were quite variable whilst i.t. instillate administration showed a more widespread distribution than in previous studies with insulin (3). It was noted that agitation of hGH solutions during their preparation produced foaming. If the protein solution had surfactant properties this may explain the higher PI values, since the inclusion of surfactants in formulations for i.t. delivery is known to improve lung distribution (9).

An advantage of combining pharmacokinetic and gamma scintigraphic methodologies is the ability to accurately determine the extent of solute deposition from an aerosol in the lung: an absolute bioavailability may be calculated with reference to i.v. pharmacokinetic data. Equivalent dose to lung values were calculated by two methods. The value determined from an attenuation factor derived following i.t. instillate administration of known amounts of radioactivity was 0.16 ± 0.08 mg kg^{-1}, whilst that calculated using perspex shields as a tissue equivalent for estimating transmittance of radiation from within the lung was 0.17 ± 0.08 mg kg^{-1}. Because of the concordance of these results, bioavail-

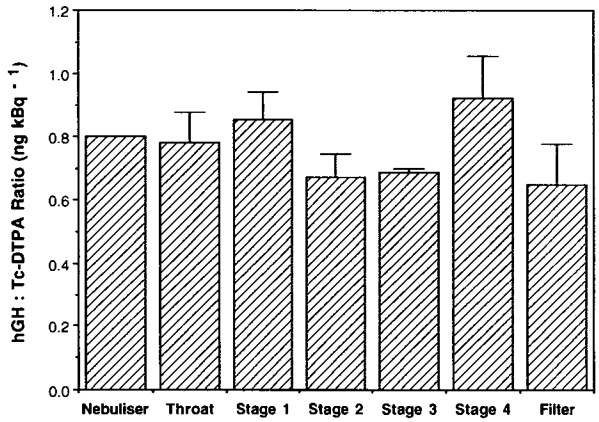

Fig. 1. Comparison of the hGH: ^{99m}Tc-DTPA ratio at each location of the MLI with that of the original nebuliser solution (mean ± SD, $n = 3$).

Table I. Human Growth Hormone Pharmacokinetic Parameters (mean ± SD, n = 5)

	i.v.	i.t. Instillate	Aerosol
CL (mL min^{-1} kg^{-1})	4.12 ± 0.57	—	—
V_{SS} (mL kg^{-1})	95.7 ± 21.7	—	—
F (%)[a]	100	15.5 ± 4.5[b]	44.8 ± 25.1[b]
k_a (min^{-1})	—	0.0012 ± 0.00048	0.00200 ± 0.00160
t_{max} (min)	—	276 ± 120	186 ± 105
k_{lb} (min^{-1})	—	0.00018 ± 0.000067[c]	0.00071 ± 0.00043[c]
k_{le} (min^{-1})	—	0.00103 ± 0.00043	0.00130 ± 0.00146

[a] F values are calculated relative to 100% after i.v. dosing.
[b,c] Significantly different ($p < 0.05$) using paired t-test.

abilities were calculated with confidence based upon the individual mean predicted doses for each animal. As reported in Table I, lower bioavailable fractions (F) were found for i.t. instillation (11.5–21.9%) compared with those following aerosol dosing (18.9–79.1%). The rate of mucociliary clearance is known to increase progressively from the peripheral to the central regions of the respiratory tract (10), therefore the rate of clearance of a slowly absorbed solute will be influenced by its site of deposition. In these studies it is likely that the extent of hGH removal by mucociliary clearance was greater following i.t. than aerosol delivery.

Plasma hGH profiles following i.v., i.t. instillate, and aerosol delivery are shown in Fig. 2. Pharmacokinetic parameters derived from these data are given in Table I. A biphasic exponential decline in plasma concentration was seen following i.v. administration. Half-lives for the initial distribution and slower elimination phases were 8.9 ± 1.6 min and 40.3 ± 7.0 min respectively (mean ± SD). These values are similar to those found in the literature for other animal models and in man (11,12). Terminal post-peak half-lives were significantly longer after aerosol (347 min) and i.t. (578 min) dosing than for i.v. ($p < 0.005$ and $p < 0.0005$ respectively). This indicated that absorption rate limited kinetics were occurring after both methods of lung delivery. Consequently post-peak half-life was used for calculating absorption rate constants. Values of k_a were statistically equivalent following aerosol and i.t. administration, which suggests that the rate of hGH absorption is independent of the mode of delivery. In previous studies, using a range of solutes (5,6) absorption rate constants following aerosol administration were reported to be roughly twice those seen after i.t. dosing. In those studies, however, mucociliary clearance was inhibited throughout the experiment. Bioavailability values from our studies suggest that mucociliary clearance or another competitive elimination process had a greater influence on the fate of the i.t. than on the aerosolized hGH. This is graphically depicted in Fig. 3 where greater central deposition (smaller PI values) for both i.t. and aerosol dosing is seen to be associated with lower bioavailable fractions.

A competitive process to absorption is well known to result in an overestimation of the true absorption rate constant (13). Therefore the equivalent values of k_a observed after i.t. and aerosol administration suggest that the true lung-to-blood transfer rate constant (k_{lb}) following i.t. delivery is likely to be lower than that following aerosol administration. The simple pharmacokinetic model shown in Fig. 4 was used to test this hypothesis. In this scheme, compounds administered to the lung are either absorbed into the bloodstream or eliminated (or irreversibly bound) by unspecified processes (e.g. mucociliary clearance and/or metabolism). The fate of compounds is then determined by two first-order rate constants; k_{lb} which controls transfer from the lung to blood and k_{le} which dictates elimination. The relative magnitudes of k_{le} and k_{lb} determine both the rate and extent of absorption of the compound into the bloodstream. The rate of absorption is reflected by an apparent (observed) absorption rate constant (k_a) which is the sun of k_{lb} and k_{le}. The extent of absorption, given by the bioavailable fraction (F), is the ratio of k_{lb}/k_a. Using Equations (1) and (2), values were

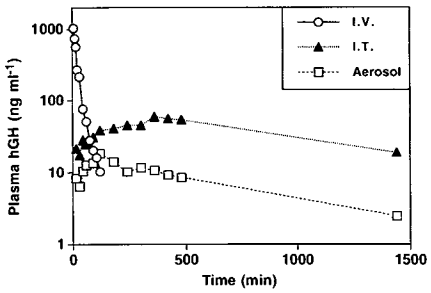

Fig. 2. Geometric mean (n = 5) plasma concentration profiles of hGH following i.v. (○), i.t. (▲), and aerosol (□) administration. Standard error bars are omitted for clarity. Refer to Table I for statistical treatment of data.

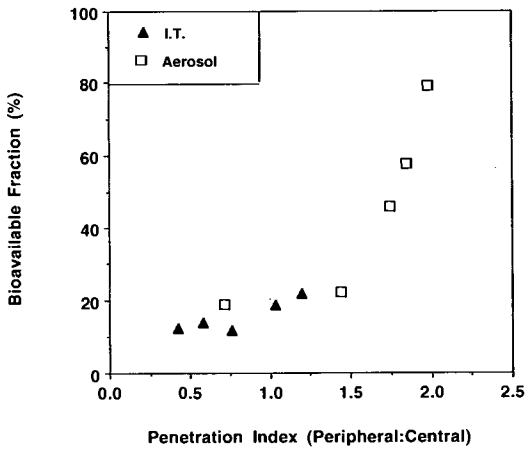

Fig. 3. The influence of penetration index (peripheral:central) on bioavailable fraction of hGH after i.t. (▲) and aerosol (□) administration.

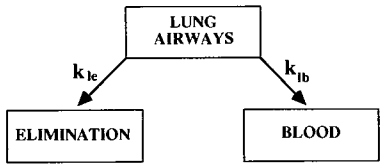

Fig. 4. A simple pharmacokinetic model of lung availability, showing rate constants for elimination from the lung (k_{le}) and transfer to blood (k_{lb}).

calculated for k_{lb} and k_{le}. These values are reported in Table I.

$$k_{lb} = F * k_a \quad (1)$$

$$k_{le} = k_a - k_{lb} \quad (2)$$

Values of k_{lb} for aerosol delivery were greater than those for i.t. dosing. This is in agreement with the hypothesis of Schanker that peripheral regions of the lung show higher permeabilities (5,6). There was no statistical difference between k_{le} values for the two modes of pulmonary delivery. This may be a result of the relatively efficient lung dispersion achieved following i.t. delivery and the variability in aerosol deposition patterns as indicated by the PI values (Fig. 3).

In summary, we have demonstrated a difference in the extent of absorption of i.t. instilled and aerosolized hGH, which seems likely to result from the differential effects of mucociliary clearance. Absorption rate constants were resolved into components for lung-to-blood transfer and elimination from the lung by mucociliary and metabolic pathways. This indicates that the permeability of lung epithelium to hGH is greater in respiratory regions than in conducting airways. Gamma scintigraphy proved a vital adjunct to the pharmacokinetic study, allowing the precise site and extent of solute deposition within the lung to be determined.

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