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Absorption of an RGD peptide (SK&F 106760) following intratracheal administration in rats

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Abstract

The objective of this study was to determine the bioavailability of an RGD peptide, SK&F 106760, following intratracheal administration in a rat model. These results provide information pertinent to determining the utility of this route for peptide delivery in general and specifically for the RGD class of peptides. The data indicate that the area under the plasma concentration versus time curve for SK&F 106760 following intratracheal administration in the rat is linearly related to dose (3 and 10 mg/kg) and bioavailability is approx. 20%. The T_{max} values after intratracheal dosing were 55.0 and 16.5 min at 3 and 10 mg/kg, respectively. By comparison, bioavailability of SK&F 106760 administered intraduodenally in the rat is approx. 0.5%. The rate of absorption varied with the pH of the dosing solution. T_{max} values decreased from 16.5 min at pH 5.5 to 4.4 min at pH 2.5 and the corresponding C_{max} increased from 2.8 to 7.8 μg/ml. However, this change in dosing solution pH had no effect on the extent of absorption. From the permeability/bioavailability data generated in this study, together with information concerning the dosage and dosage regimen required, rational decisions regarding formulation approaches and selection of route of administration can be made. These data, together with reports from the literature, clearly demonstrate that absorption of peptides via the lung may be a viable option for systemic delivery.

Key words: RGD; SK&F 106760; Peptide; Absorption; Pulmonary administration; Pharmacokinetics

1. Introduction

Increasing interest in the pulmonary route for systemic delivery of peptides and proteins has been generated by studies demonstrating absorption of a range of molecules including growth hormone, renin inhibitors, leuprolide, 1-deamino-cysteine-8-arginine vasopressin, insulin, granulocyte colony stimulating factor and the growth hormone releasing peptide, SK&F 110679 (His-

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Abbreviations: AUC, area under the plasma concentration vs time curve; %EAUC, % of total AUC that was obtained by extrapolation; i.t., intratracheal; i.v., intravenous; Vd_β, volume of distribution of elimination phase; F, systemic availability; β, elimination rate constant; k_{abs}, absorption rate constant; T_{max}, time required to achieve C_{max}; C_{max}, maximum plasma concentration; $t_{1/2\beta}$, elimination half-life; RGD, arginine-glycine-aspartic acid.

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D-Trp-Ala-Trp-D-Phe-Lys-NH_2) (Wigley et al., 1971; Elliot et al., 1987; Hubbard et al., 1989; Adjei and Garren, 1990; Adjei et al., 1990; Folkesson et al., 1990; Patton et al., 1990; Colthorpe et al., 1991; Rosenfeld et al., 1991; Sheehy et al., 1991; Laube et al., 1992; Smith et al., 1994). Aerosol delivery from either solutions or dry powders, is advantageous over oral administration in that drugs absorbed from the lung are not subject to hepatic first pass elimination or the myriad of enzymatic and chemical degradation processes present in the lumen of the gastrointestinal tract. However, a disadvantage of the aerosol route for systemic delivery is that with currently available devices, a maximum of approx. 20% of the administered dose is deposited at the presumed site of absorption (e.g., the alveolar region) (Byron, 1990). As discussed previously (Gupta and Hickey, 1991), deposition of aerosolized particles in the respiratory tract is influenced by a number of factors including physical properties (e.g., particle size and size distribution, shape and density), biological factors (e.g., vital capacity, breath-holding) and the device employed. Thus, despite relatively greater absorption via the lung compared to the intestine (Wigley et al., 1971; Elliot et al., 1987; Hubbard et al., 1989; Adjei and Garren, 1990; Adjei et al., 1990; Folkesson et al., 1990; Patton et al., 1990; Colthorpe et al., 1991; Rosenfeld et al., 1991; Sheehy et al., 1991; Laube et al., 1992; Smith et al., 1994), advances in development of formulations and/or devices to increase deposition will be required to increase the range of molecules which can be considered for delivery by this route.

To determine the permeability of the pulmonary epithelium to peptides and proteins, methods have been established for direct administration of a solution into the lung (Raeburn et al., 1992). The major advantage of direct administration compared to aerosolization is the ease with which absorption studies in animals can be conducted resulting in an increased ability to evaluate the absorption of a larger range of molecules without being concerned about particle size, stability upon nebulization and losses due to the device employed. The finding that the absorption rate constant for insulin was the same whether it was administered by intratracheal instillation of a solution or by nebulization supports the use of this approach (Colthorpe at al., 1991). SK&F 106760 (Ac-cyclo-*S*,*S*-[Cys-(N^{α}-Me)-Arg-Gly-Asp-Pen]-NH_2), is an RGD peptide which has been investigated for its potential as a fibrinogen receptor antagonist (Samanen et al., 1991). The oral bioavailability of SK&F 106760 is ~ 0.5% when administered intraduodenally in the rat (Constantinides et al., 1993). In the present study, the apparent bioavailability of SK&F 106760 after intratracheal administration has been determined with solutions having pH values of 2.5 (at 10 mg/kg) and 5.5 (at 3 and 10 mg/kg) to examine the dose dependence and effect of pH on pulmonary absorption of SK&F 106760.

2. Materials and methods

2.1. Chemicals

SK&F 106760 (Ac-cyclo-*S*,*S*-[Cys-(N^{α}-Me)-Arg-Gly-Asp-Pen]-NH_2) was obtained from Peptidomimetic Chemistry, (SmithKline Beecham Pharmaceuticals, King of Prussia, PA) dissolved in 0.9% saline solution (final pH 2.5) or Dulbecco's phosphate-buffered saline (DPBS, 21 mM phosphate, pH 5.5 without calcium or magnesium). SK&F 106673 (N^{α}-Ac-[cyclo-*S*,*S*]-Cys-Arg-Gly-Asp-Pen-NH_2, was obtained from Peptidomimetic Chemistry (SmithKline Beecham Pharmaceuticals). Sodium chloride, dextrose and pentobarbital were obtained from Abbott Labs. (North Chicago, IL). Rompun and Ketaset were obtained from Haver Moley Corp. (Shawnee, KS) and Aveco Co., Inc. (Fort Dodge, IA), respectively. Heparin was from Elkins-Sinn, Inc. (Cherry Hill, NJ). DPBS was from Gibco Life Technology, Inc. (Grand Island, NY).

2.2. Jugular catheterization

Male Sprague-Dawley rats (virus antibody free, Charles River, Raleigh, NC) weighing 300–370 g were anesthetized with Rompun (5 mg/kg) and Ketaset (35 mg/kg) diluted with 0.9% saline to a final volume of 1 ml and injected intraperi-

toneally. Upon verification of loss of pain reflex, the neck was shaved. A 2–3 cm incision was made on the ventral surface of the neck above the clavicle. Blunt dissection was employed to clear connective tissue to expose the jugular vein and sutures were placed under the vein at points proximal and distal to the clavicle. An incision was made in the jugular vein and Silastic tubing (Dow Corning, Midland, MI, 0.020 inch inside diameter) was inserted approx. 2.5 cm in the proximal section and secured with suture. A trocar was employed to provide an incision through the nape of the neck through which the catheter was inserted. The trocar was removed leaving the catheter exposed and the incision closed with wound clips. The catheter was then filled with 50% dextrose containing heparin (200 U/ml) and plugged with a stainless-steel pin. Approx. 18 h prior to experimentation, food was removed (free access to water).

2.3. Intravenous administration of SK&F 106760

Each animal was dosed via the lateral tail vein with 3 mg/kg SK&F 106760. Blood samples (0.5 ml aliquots) were collected at 0, 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min. The 0 min sample was collected 15 min prior to administration of the dose. Plasma was removed from the samples after centrifugation at $1600 \times g$ for 5 min, and stored at -20°C in 250 μl aliquots per sample. The blood pellet was then reconstituted with 12.5 U heparinized saline (volume equal to the plasma removed) and returned to the appropriate animal via the jugular catheter. At the end of the study, animals were euthanized with an overdose of pentobarbital administered intravenously and then were exsanguinated.

2.4. Intratracheal administration of SK&F 106760

Rats were anesthetized with intraperitoneal Rompun (5 mg/kg) and Ketaset (35 mg/kg) diluted with 0.9% saline to a final volume of 1 ml. Upon verification of loss of pain reflex, the rat was laid on its back and an incision ($\sim$ 1 cm) was made on the ventral surface of the neck directly above the sternum. Blunt dissection was employed to clear connective tissue and the salivary glands were gently separated. The two halves of the sternohyoid muscle thus exposed were separated and retracted laterally exposing the trachea. The dose was delivered in solution (either 0.9% saline or DPBS, 0.5 ml/kg) employing a 27 gauge needle followed by 2 ml of air to prevent fluid from being trapped in the trachea. Upon removal of the needle, a drop of surgical glue was placed at the injection site and the incision closed with surgical clips. Following dosing, rats were placed in a Braintree restraining cage (the rats regained consciousness within 10 min post dosing) for the duration of the experiment. Blood samples in intratracheal experiments (0.5 ml) were obtained at 0, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min (pH 2.5, intratracheal only) and 300, and 360 min (pH 5.5, intratracheal only). Blood samples were centrifuged and 250 μl of plasma were removed and stored at -20°C for HPLC analysis. After removal of plasma, blood samples were reconstituted with 250 ml of 12.5 IU/ml heparinized saline and reinjected via the jugular catheter to the appropriate rat. At the end of the experiment, rats were killed with an intravenous injection of pentobarbital (overdose) and exsanguinated.

2.5. Post-column HPLC fluorescence assay

Plasma concentrations of SK&F 106760 were determined using an assay procedure previously reported (Hiraga and Kinoshita, 1981; Rhodes and Boppana, 1988). Plasma samples and standards were prepared using 250 μl aliquots. SK&F 106673 (0.175 μg) was added to each sample and standard as an internal standard. Samples and standards were precipitated with 0.6 ml acetonitrile (HPLC reagent grade, J.T. Baker, Phillipsburg, NJ) and then centrifuged at $16\,000 \times g$ for 20 min. The supernatant was removed and dried under N_2 at 40°C. Pellets were redissolved in 0.5 ml ultrapure water and processed by solid-phase extraction (SPEP) on the SPEED WIZ (Model 6020, Applied Separations, Inc., Allentown, PA). SPEP was as follows: (1) columns (3 ml, Bakerbond C18, J.T. Baker, Phillipsburg, NJ) were conditioned with 4 ml methanol and rinsed with 4

ml ultrapure water; (2) standards and samples were applied to the columns and washed through with 7.4 ml of ultrapure water; (3) columns were then washed with two 1.0 ml aliquots of methanol which were collected for analysis. Samples and standards were dried under N_2 at 40°C and then dissolved in 200 μl of 10% methanol : 90% ultrapure water.

2.6. Pharmacokinetic analysis

The area under the plasma concentration vs time curves (AUC) from time = 0 to time = t ($AUC_{0\rightarrow t}$) was calculated according to the linear trapezoidal method (Gibaldi and Perrier, 1982). The extrapolated AUC ($AUC_{t\rightarrow\infty}$) was calculated by dividing the last plasma concentration (C_n) by the elimination rate constant, β (Gibaldi and Perrier, 1982).

Systemic availability, F, the fraction of dose that reaches the systemic circulation, was calculated using the equation:

$$F = (AUC_{i.t.}/AUC_{i.v.}) \times (Dose_{i.v.}/Dose_{i.t.}) \quad (1)$$

where $AUC_{i.t.}$ is the AUC following administration to the lungs intratracheally and $AUC_{i.v.}$ denotes the AUC following intravenous administration of SK&F 106760.

Systemic plasma clearance, Cl_s, and volume of distribution, Vd_β, were calculated from the intravenous data using the following equations:

$$Cl_s = Dose_{i.v.}/AUC_{i.v.} \quad (2)$$

$$Vd_\beta = Dose_{i.v.}/\beta \cdot AUC_{i.v.} \quad (3)$$

Individual plasma concentrations vs time profiles generated from i.t. dosing were modeled by employing a one-compartment model with first-order input assuming linear elimination. The absorption constant, k_{abs}, for i.t. dosing was estimated using non-linear least-squares regression analysis (PCNONLIN) (Metzler and Weiner, 1989). The maximum plasma concentration, C_{max}, and time to achieve the maximum plasma concentration, T_{max}, were obtained from the average of the maximum measured plasma concentrations and the average time at which these values were determined.

3. Results

3.1. Intravenous (i.v.) administration

Average plasma concentrations of SK & F106760 after i.v. administration of a 3 mg/kg dose are presented in Fig. 1. The total area under the plasma concentration vs time curve ($AUC_{0\rightarrow\infty}$) was 0.64 ± 0.082 mg min ml^{-1} (mean ± SD, n = 7). Systemic plasma clearance was 4.7 ml kg^{-1} min^{-1}. Volume of distribution (Vd_β) and elimination half-life ($t_{1/2\beta}$) were 201.1 ± 26.5 ml/kg and 27.3 ± 12.2 min, respectively.

3.2. Intratracheal (i.t.) administration

SK&F 106760 was rapidly absorbed after i.t. administration with the solution pH of 2.5 or 5.5 and at both 3 and 10 mg/kg doses (Fig. 2). At pH 5.5 the absorption rate constants (k_{abs}) were 0.122 ± 0.103 min^{-1} (mean ± SD, n = 6) (absorption $t_{1/2}$ = 5.7 min) and 0.231 ± 0.090 min^{-1} (n = 4) (absorption $t_{1/2}$ = 3.0 min) at 3 and 10 mg/kg, respectively. Maximum plasma concentrations, C_{max}, the time to achieve this concentration, T_{max}, and AUC are listed in Table 1. Due to the rapid achievement of maximum plasma concentrations with pH 2.5 solutions, there was insufficient data on the absorption phase to esti-

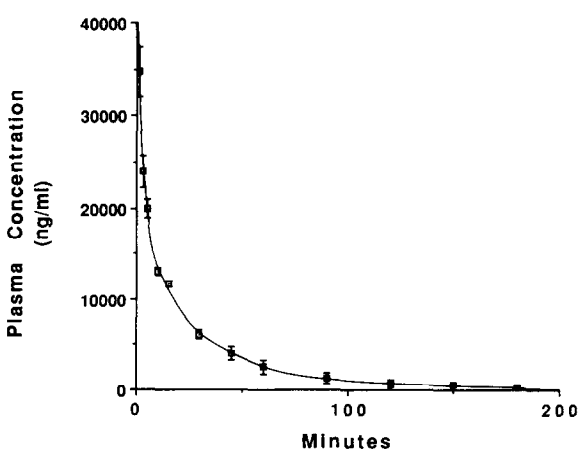

Fig. 1. Mean plasma concentration of SK&F 106760 following intravenous administration (3 mg/kg) in male rats. Results are means ± SE for four animals.

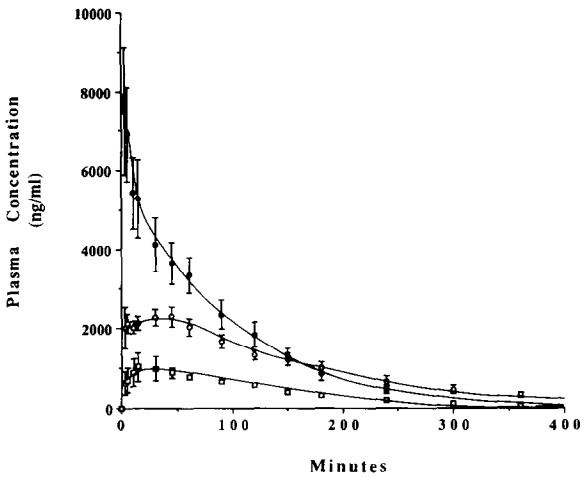

Fig. 2. Mean plasma concentrations of SK&F 106760 following intratracheal administration (10 mg/kg, pH 2.5, $n = 12$ (●); 3 mg/kg, pH 5.5, $n = 6$ (○); 10 mg/kg, pH 5.5, $n = 6$ (□)) in male rats. Results are means ± SE.

mate the absorption rate constant. At pH 2.5 the time required to achieve the maximum plasma concentration, T_{max}, following administration of 10 mg/kg was approx. 4.4 min and at pH 5.5 T_{max} values following administration of 3 and 10 mg/kg were 55.0 and 16.5 min, respectively.

Bioavailability, a measure of the rate and extent at which the compound reaches the systemic circulation, was calculated from the total AUC (e.g., $AUC_{0\to\infty}$ = the sum of the measured $AUC_{0\to t}$ plus the $AUC_{t\to\infty}$). Extrapolated AUC (e.g., C_n/β) can only be accurately calculated when the slope of the terminal phase of the plasma concentration vs time curves describes elimination alone and not elimination plus absorption. If absorption from the lung is slow, the slope of the terminal phase of the plasma concentration vs time curve will yield an apparently slower elimination rate constant than that obtained from i.v. studies. This would result in a higher apparent bioavailability. The percent extrapolated AUC for intravenous dosing was 2.1%. For intratracheal dosing, percent extrapolated AUC values were: 3.1%, at pH 2.5 (10 mg/kg); 1.5%, at pH 5.5 (3 mg/kg) and 2.8% at pH 5.5 (10 mg/kg) when calculated using the elimination constant from i.v. studies and 6.1, 8.4 and 16.9%, respectively, when determined using the elimination rate constants from i.t. studies (0.0138 min^{-1} at pH 2.5; 0.0050 min^{-1} at pH 5.5, 3 mg/kg; and 0.0049 min^{-1} at pH 5.5, 10 mg/kg). The bioavailabilities of SK&F 106760 following intratracheal dosing based on the total AUC values were 25.8 and 29% (pH 2.5), 23.4 and 25.1% (pH 5.5, 3 mg/kg) and 20 and 24.8% (pH 5.5, 10 mg/kg) with the lower values calculated using the i.v. elimination rate constant (0.0285 min^{-1}) and the higher values determined using the apparent elimination rate constant derived from the corresponding intratracheal studies (Table 1).

4. Discussion

For therapeutic agents which are peptides and proteins, instability and poor permeability characteristics in the gastrointestinal tract may preclude the use of this route. For this reason, alternative

Table 1
Bioavailability of SK&F106760 following intratracheal administration to rats at pH 2.5 vs 5.5

Route/dose (mg/kg)	T_{max} (min)	C_{max} (mg/ml)	Total $AUC_{0\to\infty}$ (mg min ml^{-1})	%EAUC	F (%)
Intravenous/3 ($n = 4$)	–	–	0.64 ± 0.08	2.1 ± 3.4	100
Pulmonary/10 pH 2.5 ($n = 12$)	4.4	7.8	0.54 ± 0.08	3.1 ± 0.4	25.8
Pulmonary/10 pH 5.5 ($n = 6$)	16.5	2.8	0.42 ± 0.04	2.8 ± 0.6	20.0
Pulmonary/3 pH 5.5 ($n = 6$)	55.0	1.2	0.15 ± 0.01	1.5 ± 0.4	23.4

Results are means ± SE for n animals. Area under the concentration vs time curve (AUC) was calculated using the trapezoidal method. Extrapolated AUC was calculated employing an elimination constant of 0.0285 min^{-1}. %EAUC, percent of the total area that was obtained by extrapolation; F, systemic bioavailability.

non-injectable methods of delivery are being investigated. The large surface area, extensive vascularity and permeability of the lung suggest that the pulmonary route may be feasible for systemic delivery of these agents. However, for the pulmonary route to be considered as a viable delivery alternative reliable methods for efficient delivery of molecules to the alveolar region will be required. To achieve deposition greater than the current 20% will require advancement in both device and formulation technologies.

Intratracheal instillation of selected compounds provides a rapid, quantifiable method of delivery for evaluation of systemic absorption via the lung. However, distribution and deposition in the lung following intratracheal instillation is highly variable (Raeburn et al., 1992) and calculation of exact doses delivered to the alveoli, the presumed absorptive region, is difficult. Additionally, the site(s) and mechanisms of absorption of peptides/proteins in the lung are not well understood. It is postulated that the alveolar region is the site of greatest absorption. This assumption is based primarily on the observations that aerosols with particle sizes less than 5 μm result in the greatest absorption and also are deposited to a greater extent in the alveolar region (Adjei and Garren, 1990). In the present study, it has been shown that the RGD peptide, SK&F 106760, is well absorbed in a dose-proportional manner following intratracheal instillation. Following administration of 3 mg/kg dose of SK&F 106760 i.t., T_{max} values were $\sim$ 1 h. This together with the fact that absorption continues for an extended period, as demonstrated by the difference in $t_{1/2}$ values obtained for i.v. and i.t. administration, suggests that plasma disposition of SK&F 106760 following pulmonary delivery is absorption rate limited (e.g., flip-flop kinetics). This phenomenon may not be unique to this compound, since a previous study found a prolonged elimination phase associated with renin inhibitors administered intratracheally (Sheehy et al., 1991). The reduction in T_{max} values at higher doses (55 min at 3 mg/kg vs 16.5 min at 10 mg/kg) cannot be explained with the available data. Comparison of the results obtained at pH 2.5 vs 5.5 indicates that the permeability of the lung to SK&F 106760 is enhanced at pH 2.5. This is demonstrated by more rapid achievement of maximal plasma concentration (4.4 vs 16.5 min) at pH 2.5 vs 5.5 although the extent of absorption is not different at pH 2.5 vs 5.5. Whether this enhanced permeability results from an effect of reduced pH on the lung epithelium (Jones et al., 1978), a change in physicochemical properties of the drug, or differences in distribution of instillate between the groups of animals cannot be distinguished from the present studies.

A recent study found that the bioavailability of insulin was 3-fold greater at pH 3.0 than at pH 7.0 following intratracheal administration in the rat (Okumura et al., 1992). The effect of pH on T_{max} was not determined in that study. However, the slope of the terminal phase at both pH values is consistent with a faster apparent elimination phase at pH 3.0. This indicates that a low pH decreases the resistance of the pulmonary epithelium to the absorption of small and large peptides. The decrease in resistance to absorption would eliminate the absortion-limited disposition of these compounds and thus eliminates the flip-flop kinetics observed at pH 5.5 in this study.

The present studies indicate that the extent of lung permeability to SK&F 106760 is much greater than that of the intestinal tract (Constantinides et al., 1993), consistent with a variety of recent studies demonstrating greater extent of absorption from the lungs than from the gastrointestinal tract (Wigley et al., 1971; Elliot et al., 1987; Hubbard et al., 1989; Folkesson et al., 1990; Adjei and Garren, 1990; Adjei et al., 1990; Patton et al., 1990; Colthorpe et al., 1991; Rosenfeld et al., 1991; Sheehy et al., 1991; Laube et al., 1992). The 20–25% absorption seen following intratracheal administration of a solution is probably an underestimate, since this administration procedure results in variable amounts of delivery to the lung. Furthermore, absorption after aerosolization is generally greater than after intratracheal administration presumably due to the more uniform distribution achieved (Colthorpe et al., 1991). Thus, absorption of the dose delivered to the lung after aerosolization may be more efficient than the 20–25% observed following intratracheal administration of a solution.

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