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The pharmacokinetics of pulmonary insulin in the in vitro isolated perfused rat lung: Implications of metabolism and regional deposition

Yinuo Pang, Masahiro Sakagami *, Peter R. Byron

Department of Pharmaceutics, School of Pharmacy, Virginia Commonwealth University, 410 North 12th Street, P.O. Box 980533, Richmond, VA 23298-0533, USA

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Abstract

The pharmacokinetics of several lung disposition pathways for pulmonary insulin were studied and modeled in the isolated perfused rat lung (IPRL). Insulin solution was administered by forced instillation into the airways of the IPRL as 0.1 or 0.02 ml doses of coarse spray, with or without bacitracin (BAC), *N*-ethylmaleimide (NEM) and atrial natriuretic peptide (ANP). Each insulin absorption profile was fitted to a kinetic model that incorporated the distribution fraction of the dose reaching the lobar region (DF) and the rate constants for absorption into perfusate (k_a) and non-absorptive loss (k_{nal}); k_{nal} was shown to be due to the sum of mucociliary clearance and metabolism. Insulin absorption occurred largely by passive diffusion with values for $k_a = 0.39–0.50\ h^{-1}$. With DF = 0.91 following 0.1 ml doses, 11.9 ± 3.4% of bioavailabilities were observed in 1 h. In contrast, derived values for $k_{nal} = 2.34–3.45\ h^{-1}$ were significantly larger than the rate constant for mucociliary clearance determined previously in this IPRL ($0.96–1.74\ h^{-1}$) due to lung metabolism. Indeed, BAC, but neither NEM nor ANP, was found to decrease the value of k_{nal}, which suggested that BAC-inhibitable lung ectopeptidases, and not insulin degrading enzyme (IDE), were responsible for this pulmonary metabolism. Shallower lung distribution with DF = 0.73 following 0.02 ml doses resulted in reduced values for $k_a = 0.27\ h^{-1}$ and $k_{nal} = 2.79\ h^{-1}$, indicating that these kinetic processes may be lung-region dependent, even within this model and emphasizing the likely importance of reliable lung deposition in vivo.

Keywords: Insulin; Lung; Deposition; Absorption; Metabolism; Mucociliary clearance; Pharmacokinetics

1. Introduction

Oral inhalation of insulin has been shown to offer non-invasive glycemic control in diabetic treatments with an earlier T_{max} (time to reach the maximum concentration) in the systemic circulation, when compared to subcutaneous injection (Patton et al., 1999a,b; Sakagami, 2004). Moreover, much of the research on advanced inhalation delivery for macromolecules has also employed this polypeptide as a precursor to launching a series of technology platforms, aimed at improvements in therapeutic outcome and/or patient compliance, e.g., long-acting and/or increased bioavailability formulations (Agu et al., 2001; Edwards and Dunber, 2002; Sakagami and Byron, 2005). However, most

Abbreviations: APN, aminopeptidase N; ANP, atrial natriuretic peptide; BAC, bacitracin; BSA, bovine serum albumin; C_{max}, the maximum serum/plasma concentration; COD, coefficient of determination; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; FD-4, fluorescein isothiocyanate (FITC)-labeled dextran 40; IDE, insulin degrading enzyme; IPRL, isolated perfused rat lung; MDI, metered dose inhaler; MSC, model selection criterion; NEM, *N*-ethylmaleimide; NIH, National Institute of Health; PBS, phosphate-buffered saline; RIA, radio immunoassay; T_{max}, the time to reach the C_{max}; USP, the United States Pharmacopeial Convention Inc.; VCU, Virginia Commonwealth University

* Corresponding author. Tel.: +1 804 828 2014; fax: +1 804 828 8359.
E-mail address: msakagam@vcu.edu (M. Sakagami).

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of those studies were based on in vivo tests in animals, which employed a variety of insulin dosing techniques, such as intratracheal instillation, microspray, nebulization, aerosol puff or exposure; these likely resulted in different regional distributions in the lung. Accordingly, they have reported substantially different and often variable insulin profiles following pulmonary administration, as we will discuss later with Table 5. Clearly, to unify these studies, it is necessary to gain a systematic understanding of the lung's handling of this polypeptide kinetically and mechanistically, especially in terms of insulin's absorption, mucociliary clearance, tissue sequestration and/or metabolism (Byron, 1986; Taylor et al., 1994; Sakagami et al., 2002a).

We have shown previously that our in vitro lung tissue preparation, the isolated perfused rat lung (IPRL), when employed with forced solution instillation and kinetic modeling, enables independent derivation of kinetic descriptors for absorption from the lobar regions and mucociliary clearance (Sakagami et al., 2002a,b). Notably, its disposition kinetics for certain macromolecules has shown to be predictive of in vivo behavior, by virtue of their insignificant absorption from the upper tracheobronchial airways (Sakagami et al., 2002a). Even so, the lung lobar dose excluding the tracheobronchial counterpart should not be solely the subject of matter defining subsequent disposition kinetics for macromolecules including insulin, although determining within-lung-lobar distribution is a quite challenging experimental task. In the mean time, insulin seems to undergo substantial metabolic degradation in the lung (Liu et al., 1992; Yamamoto et al., 1994; Hsu and Bai, 1998), which must be a parallel competitive process to absorption and mucociliary clearance. As discussed above, each of these simultaneous disposition processes should depend on lung regional distribution. It is obvious, therefore, that we can benefit from the performance of well-controlled studies like the IPRL, alongside the appropriate modeling approach, so that each of the complex lung disposition pathways could be kinetically delineated and mechanistically clarified, as their independent assessments have been quite challenging and indeed, often unfeasible in previous studies in the literature.

Hence, this study continued to model lung disposition kinetics in the IPRL using metabolically susceptible insulin. Insulin absorption profiles in the absence or presence of lung disposition modifiers were characterized in the IPRL following forced instillations of 0.1 and 0.02 ml insulin solutions that were found to cause different lung distribution fractions reaching the pulmonary lobar region (DF). Subsequently, each of the profiles was curve-fitted to a single kinetic model that incorporated such a lung distribution factor (DF) alongside absorption, mucociliary clearance and metabolism. By doing so, we could derive the kinetic descriptors (i.e., rate constants) for each of the lung-region-specific disposition processes, at defined, yet different lung regional distributions.

2. Materials and methods

2.1. Materials

Humulin® (regular human insulin injection; Eli Lilly and Company, Indianapolis, IN) was purchased through the Central Pharmacy of the Virginia Commonwealth University (VCU) Health System. This formulation contains human zinc insulin at 100 IU/ml dissolved in a non-buffered aqueous system, alongside glycerol and *m*-cresol (Richards et al., 1998). A reference marker solute, fluorescein isothiocyanate (FITC)-labeled dextran (FD-4; weight-averaged molecular weight = 4.3 kDa; FITC content = 0.004 mol per glucose monomer unit) as well as bovine serum albumin (BSA; powder; fraction V; minimum 98%), bacitracin (BAC; 70,000 U/g), *N*-ethylmaleimide (NEM) and atrial natriuretic peptide (ANP, rat) were obtained from Sigma–Aldrich (St. Louis, MO). Other chemicals used to prepare Krebs–Henseleit buffer (pH 7.4) and 0.05 M phosphate-buffered saline (PBS; pH 7.4), i.e., D-glucose, KCl, $MgSO_4 \cdot 7H_2O$, $NaHCO_3$, $CaCl_2 \cdot 2H_2O$, NaCl, KH_2PO_4, $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 7H_2O$, were all purchased from Fisher Scientific (Pittsburgh, PA); these solutions were prepared, as described previously (Sakagami et al., 2002a).

2.2. Animals

Specific pathogen free, male Sprague–Dawley rats weighing 300–325 g were received from Hilltop Laboratory Animals Inc. (Scottsdale, PA) and housed in rooms controlled between 20–25 °C and 40–70% relative humidity with dark–light cycling every 12 h. The animals had free access to food and water during acclimation for >2 days prior to experimentation. All animal experiments and the protocols described below adhered to the NIH Principles of Laboratory Care and were approved by the VCU Institutional Animal Care and Use Committee.

2.3. In vitro isolated perfused rat lung (IPRL) studies

The IPRL preparation and the dosing method, forced solution instillation, were developed and carefully controlled in our laboratory, as described in detail previously (Byron and Niven, 1988; Niven et al., 1990; Byron et al., 1994; Sun et al., 1999; Sakagami et al., 2002a,b). These were used unchanged, except for the use of 0.1 and 0.02 ml volumetric doses for instillation. Briefly, a rat lung was surgically removed and housed horizontally in an artificial thorax maintained at 37 °C. Krebs–Henseleit buffer containing 4% (w/v) BSA was used as perfusate and re-circulated through the pulmonary circulation at a constant flow rate of 15 ml/min. Insulin dosing solutions were prepared by diluting Humulin® with PBS to produce concentrations of 1.5, 3.0 and 15.0 IU/ml (≈0.06, 0.12 and 0.6 mg/ml, respectively, assuming 1 mg = 26 IU of minimum potency according to USP). FD-4 solutions were