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Assessment of transport rates of proteins and peptides across primary human alveolar epithelial cell monolayers

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ABSTRACT

In this study, we investigated bi-directional fluxes (i.e., in absorptive and secretive directions) of human serum proteins [albumin (HSA), transferrin (TF), and immunoglobulin G (IgG)] and peptides/proteins of potential therapeutic relevance [insulin (INS), glucagon-like peptide-1 (GLP-1), growth hormone (GH), and parathyroid hormone (PTH)] across tight monolayers of human alveolar epithelial cells (hAEpC) in primary culture. Apparent permeability coefficients (P_{app}; $\times 10^{-7}$ cm/s, mean ± S.D.) for GLP-1 (6.13 ± 0.87 (absorptive) versus 1.91 ± 0.51 (secretive)), HSA (2.45 ± 1.02 versus 0.21 ± 0.31), TF (0.88 ± 0.15 versus 0.30 ± 0.03), and IgG (0.36 ± 0.22 versus 0.15 ± 0.16) were all strongly direction-dependent, i.e., net absorptive, while PTH (2.20 ± 0.30 versus 1.80 ± 0.77), GH (8.33 ± 1.24 versus 9.02 ± 3.43), and INS (0.77 ± 0.15 versus 0.72 ± 0.36) showed no directionality. Trichloroacetic acid precipitation analysis of tested molecules collected from donor and receiver fluids exhibited very little degradation. This is the first study on permeability data for a range of peptides and proteins across an in vitro model of the human alveolar epithelial barrier. These data indicate that there is no apparent size-dependent transport conforming to passive restricted diffusion for the tested substances across human alveolar barrier, in part confirming net absorptive transcytosis. The obtained data differ significantly from previously published reports utilising monolayers from different species. It can be concluded that the use of homologous tissue should be preferred to avoid species differences.

1. Introduction

Although immunocytochemical and biochemical approaches have been used to demonstrate the presence of serum proteins (e.g., albumin, transferrin, cerulo-plasmin, and immunoglobulin G) in bronchoalveolar lavage fluid and respiratory tract of lungs of various animal species, the rates and modes for traversing of proteins across the alveolar epithelium remain an issue of debate (Kim and Malik, 2003; Hastings et al., 2004). In a related subject matter, the lung is considered as a potential

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Table 1 – Molecular weight, isoelectric point (IEP), and apparent permeability coefficient (P_{app}) of tested proteins and peptides across hAEpC monolayers

	Molecular weight (Da)	IEP	P_{app}, a-to-b	P_{app}, b-to-a	P_{app}, a-to-b, rat AEpC	P_{app}, b-to-a, rat AEpC
GLP-1(7–37)	3355	4.9	6.13 ± 0.87 (n = 12)	1.91 ± 0.51 (n = 12)	na	na
PTH(1–38)	4458	8.6	2.20 ± 0.30 (n = 10)	1.80 ± 0.77 (n = 11)	na	na
Insulin	5800	5.4	0.77 ± 0.15 (n = 8)	0.72 ± 0.36 (n = 8)	0.21 ± 0.02 (n = 3)	0.12 ± 0.02 (n = 3)
Growth hormone	22125	5.0	8.33 ± 1.24 (n = 8)	9.02 ± 3.43 (n = 12)	0.041 ± 0.002 (n = 3–6)	0.074 ± 0.013 (n = 3–6)
Albumin	65000	4.9	2.45 ± 1.02 (n = 10)	0.21 ± 0.31 (n = 8)	0.77 ± 0.32 (n = 3)	0.39 ± 0.01 (n = 3)
Transferrin	76500	5.9	0.88 ± 0.15 (n = 8)	0.30 ± 0.03 (n = 9)	1.10 ± 0.35 (n = 3)	0.47 ± 0.02 (n = 3)
IgG	150000	5.8–7.3	0.36 ± 0.22 (n = 8)	0.15 ± 0.16 (n = 8)	0.91 ± 0.06 (n = 3)	0.17 ± 0.09 (n = 3)

Data appearing in the last two columns for rat AEpC monolayers are taken from Matsukawa et al. (2000), Yamahara et al. (1994), and Bosquillon et al. (2004b). All P_{app} values are expressed as mean ± S.D. (cm/s) × 10^{-7}. The abbreviations of a-to-b and b-to-a denote the apical-to-basolateral and basolateral-to-apical directions, respectively. na: not available.

alternative route for the systemic delivery of biopharmaceuticals (i.e., proteins and peptides) (Byron and Patton, 1994; Paul et al., 2005). In this study, we investigated proteins and peptides with molecular weights (M_W) ranging from 3300 to 150,000 Da (Table 1) in bi-directional transport studies across an in vitro model of the human alveolar barrier.

The molecules tested in this study are briefly described hereafter. Glucagon and related peptides constitute a family included in the proglucagon molecule, which has the identical sequence in the pancreas, intestine and brain. In gut L-cells, the C-terminal portion of proglucagon is predominantly processed to glucagon-like peptide-1 (GLP-1). Further processing produces the truncated and amidated forms of the peptide: GLP-1(1–36) amide (M_W 4111 Da), GLP-1(7–36) amide (M_W 3297 Da), and GLP-1(7–37) (M_W 3355 Da), which all retain biological activity. Upon binding to its receptor, GLP-1 stimulates insulin secretion in a glucose-dependent manner (Richter et al., 1990). Parathyroid hormone (PTH) is secreted by the parathyroid glands and is a major mediator of calcium and phosphate metabolism through its interactions with receptors in kidney and bone. It appears to be a protein containing 84 amino-acid residues, a sequence of which about 33–35 are necessary for biological activity. PTH(1–34) was reported with an absolute bioavailability of ~34% in in vivo rat lung studies, making it a promising candidate for pulmonary drug delivery (Codrons et al., 2003, 2004). Insulin (INS) is a two-chain polypeptide hormone of 5800 Da produced by the β-cells of pancreatic islets. It regulates the cellular uptake, utilisation, and storage of glucose, amino acids, and fatty acids and inhibits the breakdown of glycogen, protein, and fat (Morishige et al., 1977). The human growth hormone (GH) is a member of the somatotropin/prolactin family of hormones which play an important role in growth control. The isoform I has 191 amino acid residues and a molecular weight of 22,125 Da. Action of GH is regulated upon binding to the growth hormone receptor (GHR) expressed at cytoplasmatic membranes of various cells and/or the soluble growth hormone binding protein (GHBP) (Allen et al., 2000). Albumin (HSA) is a soluble, monomeric protein which comprises about one-half of the blood serum protein. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilising extracellular fluid volume (Kim et al., 2003). It is a globular unglycosylated serum protein of a molecular weight 65,000 Da. Albumin is synthesised in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted form of albumin. Transferrin (TF) is a glycoprotein of an approximate molecular weight of 76,500 Da. It transports iron from the intestine, reticuloendothelial system, and liver parenchymal cells to all proliferating cells in the body. In addition to its function in iron transport, this protein may also have a physiologic role as granulocyte/pollen-binding protein (GPBP) involved in the removal of certain organic matter/allergens from serum (Widera et al., 2003a). Immunoglobulin G (IgG) antibody molecules have biological properties conferred by transport across the maternal–foetal membranes, interaction with the classical complement system, and fixation to heterologous tissues via the Fc fragment of IgG. IgG molecules have a molecular weight of ~150,000 Da (Spiekermann et al., 2002).

Although we know peptides and proteins get transported across the rodent alveolar epithelium via various mechanisms, information specific to human alveolar epithelium is lacking to date (Kim and Malik, 2003). Thus, in this study, we measured in vitro permeability characteristics of a series of compounds across monolayers of primary cultured human alveolar epithelial cells (hAEpC) grown on tissue culture-treated filter inserts. These monolayers comprise alveolar epithelial type I-like cells and develop a high barrier resistance (>1000 Ω cm^2) (Elbert et al., 1999; Ehrhardt et al., 2005). We found that there is no apparent size-dependent transport conforming to passive restricted diffusion for serum proteins (HSA, TF, and IgG) across human alveolar barrier, in part confirming the net absorptive transcytosis of these macromolecules. Some of the investigated therapeutic proteins and peptides (PTH, GH, and INS) appear to be passively transported, while GLP-1 exhibited a significant net absorption.

2. Materials and methods

2.1. *Peptides and proteins*

Unlabelled human recombinant insulin, fluorescein isothiocyanate (FITC)-labelled human serum albumin (HSA) and FITC-labelled human immunoglobulin G were all obtained