Toxicological Characterization and Efficacy of Inhaled Custom Peptides

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ABSTRACT

In recent years, a number of new therapeutic peptides and proteins have been developed, but they must be injected in order to avoid rapid degradation by gastrointestinal enzymes. Significant development in new drug delivery systems such as nasal and lung delivery has been growing. Unfortunately, new therapeutic macromolecules are too large to pass through the epithelial and endothelial barriers without an absorption enhancer. A preliminary experiment was performed to determine the toxicity and efficacy of a Custom Peptide when administered by inhalation exposure once daily for 5 consecutive days to Sprague-Dawley rats. The proposed use of the inhaled Custom Peptide would introduce an excipient that can be used to enhance nasal and pulmonary drug delivery in tandem with a variety of inhaled pharmaceuticals. The results of this experiment demonstrated that dose levels up to 26.0 mg/kg/day did not produce any adverse test article-related findings. The Custom Peptide was well tolerated and there were no adverse clinical observations or systemic effects detected by assessment of clinical pathology parameters or following histopathological examination of all major organs. The respiratory tract (nasal cavity, nasopharynx, larynx, trachea, carina, bronchi and lungs) was examined histologically and there were no indications of local toxicity. The stability of the Custom Peptide in dose formulation solutions (2, 10, 50 mg/ml) was determined in vitro by assessing the biological activity on epithelial cell-cell adhesion. The Custom Peptide was capable of disrupting adhesion of confluent epithelial cells.

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INTRODUCTION

Nose and lung tissues represent very large surface areas of the human body that are highly vascularized ([6]Rhoades & Tanner, 2003). These tissues therefore have tremendous potential as a route for systemic delivery of therapeutic agents. Some of the most promising therapeutic agents are peptides and proteins, which could be administered by inhalation instead of by injection, thereby improving compliance ([1]Adjei & Gupta, 1997). Today's delivery systems can enable reliable and efficient deposition on all different epithelial surfaces in the nose and lung for potential absorption. Unfortunately, epithelial and endothelial barriers limit the paracellular transport of drugs. These barriers are caused by tight junctions (TJs) formed between either epithelial or endothelial cells (Figure 1). The TJs must be breached in order to get drugs into the systemic circulation via the nose or lungs. The permeability of a drug can be improved by a permeation enhancer, which will transiently open the intercellular junctions ([2]González-Mariscal et al, 2005). The adherens junctions (AJs) (Figure 2) are composed of classical cadherins that are required to initiate cell-cell adhesion and subsequently the formation of TJs that regulate paracellular transport ([4]Nelson, 2003). In the epithelium, epithelial (E)-cadherin is the major component of AJs. This cadherin regulates the formation and stability of AJs, as well as of TJs (Tunggal et al, 2005). In the endothelium, neural (N)-cadherin is involved in blood vessel formation and maintenance ([5]Gerhardt and Betsholtz, 2003; [3]Paik et al., 2004). N-cadherin also indirectly regulates the formation of endothelial TJs. E- and N-cadherin antagonists should therefore be useful in enhancing drug uptake by increasing the permeability of the epithelium and endothelium, respectively.

A preliminary experiment was performed to determine the toxicity and efficacy of a Custom Peptide, a new E- and N-cadherin antagonist, (formulated in sterile water) when administered by inhalation exposure once daily for 5 consecutive days to Sprague-Dawley rats. This experiment focused on the projected mechanisms and efficacy of the Custom Peptide acting as an absorption enhancer across the epithelial and endothelial barriers in the lungs. In addition, the local and systemic toxicity was evaluated based on clinical observations, clinical pathology and histopathology endpoints. The stability of the Custom Peptide in dose formulation solutions (2, 10, 50 mg/ml) was determined in vitro by assessing the biological activity on epithelial junctions using confluent MDCK cell cultures which were incubated in the presence of the Custom Peptide.

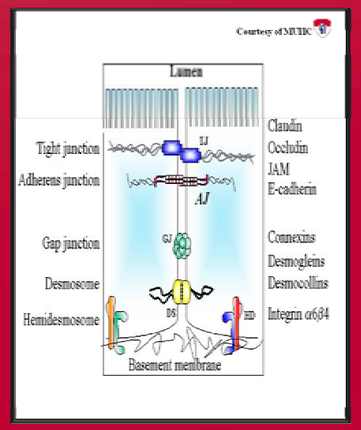

Figure 1: Epithelial intercellular junctions consist of unique protein complexes. They are essential for adhesion between opposing cells and for cell attachment to the basement membrane. Tight junctions control paracellular transport of macromolecules.

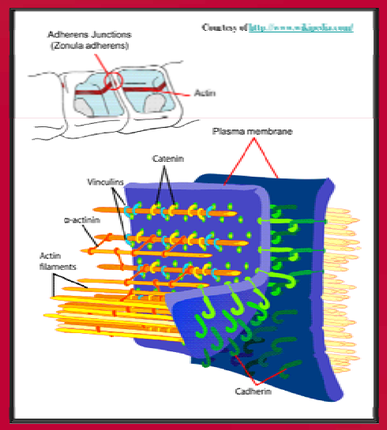

Figure 2: Principal interactions of structural proteins at cadherin-based adherens junction. Actin filaments are linked to α-actinin and to membrane through vinculin. The head domain of vinculin associates to E-cadherin via α-, β - and γ -catenins. The tail domain of vinculin binds to membrane lipids and to actin filaments.

EXPERIMENTAL DESIGN

Aqueous formulations of Custom Peptide were prepared by mixing the powder with sterile water for injection USP to achieve concentrations of 2, 10, 50 and 50 mg/ml for Groups 2, 3, 4 and 5, respectively (Table 1). Aerosols were produced by metering the flow of the Custom Peptide /control solutions with a syringe pump to clinical nebulizers (Sidestream) connected to high velocity airstreams. The aerosol produced was discharged into a flow-past inhalation exposure system (Figure 3). Doses for all groups were delivered as a 60-minute inhalation of generated aerosols on a daily basis for 5 days. Measured aerosol particle size data (MMAD = 0.3 – 0.9 µm) indicated that the aerosols were respirable. The toxicology animals in Groups 1 to 4 (including Toxicokinetic animals from Groups 1 and 4) were euthanized upon completion of the treatment period. All animals in Groups 5 were euthanized approximately post 1 hr exposure (within 10 minutes after exposure) in order to establish the ability of the Custom Peptide to induce a biological response in the target tissues (nasal cavity and the lungs).

An aliquot of each dose formulation solution (2, 10, 50 mg/ml) was used to assess the Custom Peptide stability by testing its biological activity on epithelial cell adhesion *in vitro*. The epithelial cells used were Madin-Darby canine kidney (MDCK) cells. MDCK cells were plated on 4 well slides and incubated for 48 hours to reach confluence. Medium or medium containing the Custom Peptide (0.5 mM) was added to the cells. After 1 h treatment, cells were examined by phase contrast microscopy. Zona Occludens-1 (ZO-1), a component of the TJs, was detected by immunofluores-cence to determine the opening of cell junctions.

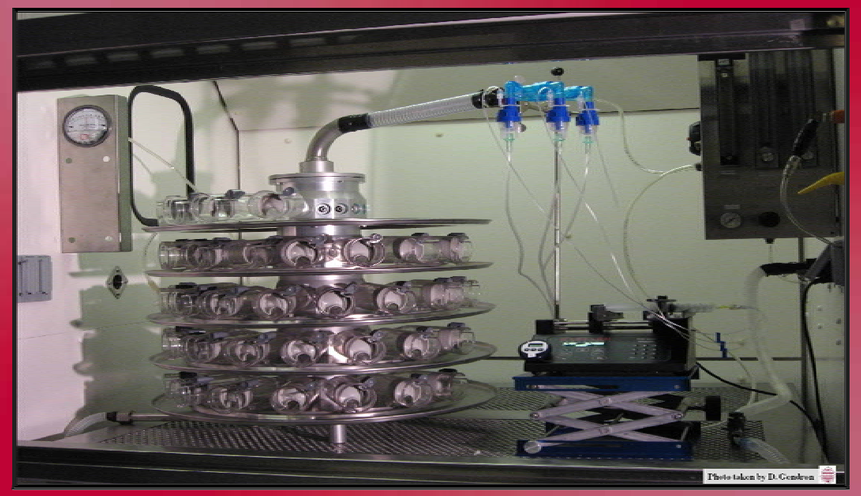

Figure 3: Flow-past inhalation exposure system with a syringe pump to clinical nebulizers (Sidestream) connected to high velocity airstreams.

Table 1: Study Design

Group Number	Group Designation	Target Dose of Custom Peptide Level (mg/kg/day) [c]	Target Aerosol Concentration of Custom Peptide (mg/L)	Formulation Concentration of Custom Peptide (mg/ml)	Toxicology Animals			
					Main Study Phase Animals		Toxicokinetic Phase Animals [c]	
					Male	Female	Male	Female
1	Control [a]	0	0 [d]	0	5	5	3	3
2	Custom Peptide Low Dose [a]	1.0	0.027	2	5	5	-	-
3	Custom Peptide Mid Dose [a]	5.2	0.133	10	5	5	-	-
4	Custom Peptide High Dose [a]	26.0	0.667	50	5	5	3	3
5	Efficacy [b]	26.0	0.667	50	5	5	-	-

[a] Group 1 - 4 main study animal and Toxicokinetic animals from Groups 1 and 4 were terminated on Day 6.
[b] This group demonstrated the ability of the Custom Peptide to induce a certain action and an affinity to bind to its receptor at a certain concentration. All main study animals from this group were sacrificed approximately post 1 hr exposure (within 10 minutes after exposure) on Day 5.
[c] Based on an estimated body weight of 0.250 kg and exposure duration of 60 minutes.
[d] Control animals were exposed to an aerosol generated from vehicle solution at an aerosol concentration considered to be equivalent in terms of mass to that generated for the High-Dose group.
[e] Due to the lack of bioanalytical testing methodology, toxicokinetic samples were not analyzed but have been stored for future analysis.

RESULTS & DISCUSSION

Aerosol administration of the Custom Peptide to the pulmonary epithelium of Sprague-Dawley rats for 5 consecutive days at doses up to 26.0 mg/kg/day was well tolerated and there were no adverse clinical observations or systemic effects detected by assessment of clinical pathology parameters or following histopathological examination of all major organs. The respiratory tract (nasal cavity, nasopharynx, larynx, trachea, carina, bronchi and lungs) was examined histologically and there were no indications of local toxicity. Based on *in vitro* testing, MDCK cell cultures incubated in the presence of Custom Peptide (0.5 mM for 1 h) in dose formulation solutions of 2, 10 and 50 mg/ml resulted in the disruption of cell adhesion (Figure 4). Furthermore, Custom Peptide altered the expression and distribution of the intercellular junction protein, ZO-1 within 1 h (Figure 5). ZO-1 was present at the adhesive interface in a continuous pattern. Treatment with the Custom Peptide caused the cell surface expression of ZO-1 to become punctuate and discontinuous. This demonstrated that the Custom Peptide had the ability to alter the epithelial barriers.

These preliminary results represent a significant opportunity for the Custom Peptide to act as an excipient and support the administration for many classes of therapeutic drugs including micro and macromolecules. However, further considerations for the preclinical development of the inhaled Custom Peptide include: an essential pharmokinetic profile to demonstrate the rapid degradation of the Custom Peptide necessary for a reversible effect, and studies on immune responses and biomarkers.

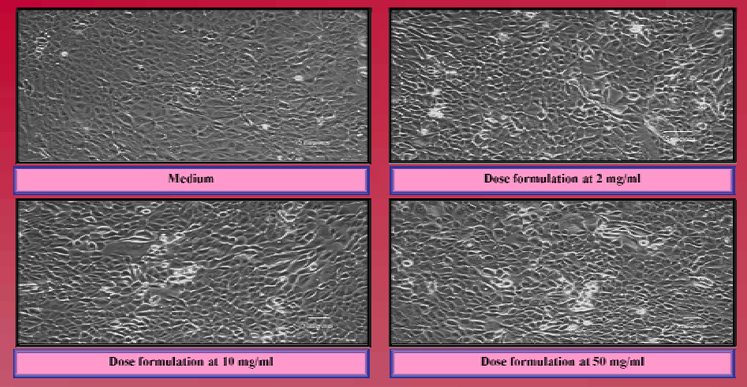

Figure 4: Phase contrast images of MDCK cell cultures treated for 1 hour with the stock solutions of Custom Peptide dose formulation (2, 10 and 50 mg/ml) at a concentration of 0.5 mM.

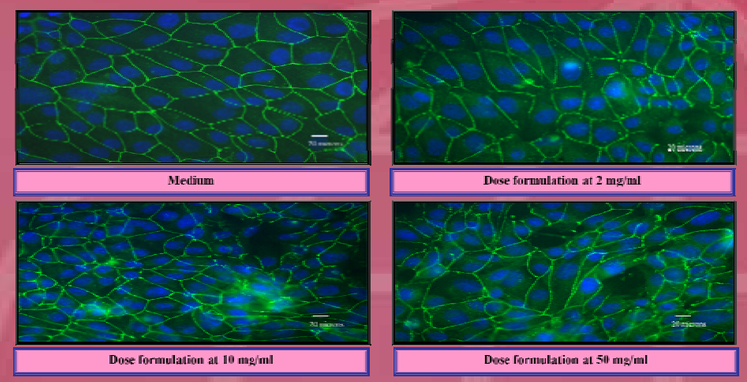

Figure 5: ZO-1 immunofluorescence of MDCK cell cultures treated for 1 hour with the stock solutions of Custom Peptide dose formulation (2, 10 and 50 mg/ml) at a concentration of 0.5 mM.

CONCLUSION

Aerosol administration of the Custom Peptide to Sprague-Dawley rats for 5 consecutive days at doses up to 26.0 mg/kg/day was well tolerated and there were no indications of local of systemic toxicities. Custom Peptide in dose formulation solutions of 2, 10 and 50 mg/ml resulted in the opening of cell junctions *in vitro*. Studies are currently being conducted to evaluate the expression of cell junction proteins on animal tissues. The results indicate Custom Peptide could be used to further enhance drug delivery across epithelial and endothelial barriers.