A Nicotinic Acetylcholine Receptor α1-specific Aptide for Potential Use in Pulmonary Drug Delivery

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ABSTRACT SUMMARY

In the pulmonary drug delivery, a major hurdle is self-cleaning mechanisms such as cough, swallowing, mucociliary movement and phagocytosis in lung. The ability of an affinity molecule to bind lung epithelium may prolong the retention of therapeutic molecules within the lung and thereby enhance the bioavailability. To this end, we screened and identified a high-affinity peptide (aptide), having very high affinity (Kₐ = 47 nM) for mouse nicotinic acetylcholine receptor α1. The high affinity and specificity were assured by Surface Plasmon Resonance (SPR) analysis and Enzyme Linked ImmunoSorbent Assay (ELISA). Upon in vivo treatment, Cy5.5-conjugated aptides showed 4 fold higher signals than Cy5.5-conjugated scrambled aptide. The results show that APTₜₐ₇₈₃₄₅₆₇₈₉ has a good potential as a conjugation partner of therapeutic molecules to increase their pulmonary retention and bioavailability.

INTRODUCTION

Lung targeted drug delivery passing through the respiratory tracts has been considered as an attractive solution for local (lung) as well as systemic therapy. It offers several advantages; First, large surface area for drug absorption (70 ~ 100 m² for human alveoli) and thin barrier between pulmonary lumen and capillary (0.1 ~ 0.2 μm for human). Second, permeable to the wide range of molecular weight, ranging from small chemical drug or peptide to large protein like IgG (>150 kDa). Third, low drug metabolizing enzymes, beneficial to enhanced bioavailability. Despite such advantages, however, there are several obstacles associated with pulmonary delivery. A major hurdle is natural cleaning mechanisms developed in the respiratory tract and lung like cough, swallowing, mucociliary clearance and phagocytosis by alveolar macrophages. In fact, a considerable amount of delivered molecules are removed by these mechanisms. Thus, it is highly demanded to overcome the rapid clearance and promote drug absorption. Herein, we report a new aptide targeting nicotinic acetylcholine receptor-α1, expressed in lung. Aptide is a peptide having high affinity to its target molecule and has a specific scaffold structure. It was screened by simple phage display technique.

EXPERIMENTAL METHODS

Biopanning using ‘Trpzip’-scaffold aptide library displayed on M13 phages was conducted and custom-synthesized biotinylated 32-amino-acid-long peptide (biotin-SGEVVIKERGWKHWVFYSCC-PTTPYLDITYH, a fragment of extracellular domain of nAChR α1; Ser193-His224) was used as a biopanning target in the form of complex with streptavidin (SA). After 5 times of biopanning procedures; target immobilization, phage library loading, washing, elution, amplification of elute phage, 40-phage clones randomly chosen from 5⁰ biopanning were applied to phage ELISA. In phage ELISA, each amplified phage clones was loaded on biotinylated nAChR α1 fragment-SA (target) and SA or BSA (control). Then, HRP conjugated anti-M13 antibody was loaded after brief washing with 0.1% PBST. Finally TMB substrate was treated for color formation. Color intensity was estimated by ELISA Reader at Abs. 450nm. Hit phage clone selected from phage ELISA was amplified and its phagemid DNA was sequenced. Cysteinylated hit aptide (named as APTₜₐ₇₈₃₄₅₆₇₈₉) and control aptide (APT₉ₓ₇) were custom-synthesized and conjugated with maleimide form of Alexa488 and cy5.5. Conjugation was done in anhydrous DMSO with stirring at room temperature for 24 h. HPLC purified conjugates were further used for in vitro and in vivo experiment. Alexa488-conjugated APTₜₐ₇₈₃₄₅₆₇₈₉ and APT₉ₓ₇ were treated to mouse nAChR α1 positive and negative cell lines or tissues (nAChR α1 positive: C2C12, MLE12 cell line and, muscle, lung; nAChR α1 negative: HEK293, CHO-K1 and spleen, kidney) for the check of specific recognition of nAChR α1.
protein in the sample and imaged using confocal fluorescence microscope. For the assessment of prolonged retention of hit aptide, 60μL-2 nM Cy5.5-conjugated APT\textsubscript{nAChR-α1} or APT\textsubscript{scr} was administered to mouse lung by tracheal injection. The lungs were dissected from each mouse at different time points (20, 50 h) and monitored using optical imaging instrument (Xenogen IVIS-100). Furthermore, harvested lungs were sliced into 8 pieces longitudinally and observed under IVIS-100.

**RESULTS AND DISCUSSION**

The amino acid sequence of hit aptide is (APT\textsubscript{nAChR-α1}); \textit{N’}-EASFWLGSWTWENGK(Cys)WT -WTWKGTKTLNR-C’ and scrambled aptide (APT\textsubscript{scr}); \textit{N’}-GGGGSGGSWTWENGK(Cys)WTWTWKGGSGG -GG-C’ (bold letters indicate the sequence of trpzip scaffold and ε-amino group of the underlined lysine is the site where a cysteine residue was adopted to conjugate with maleimide dye). To evaluate the kinetic parameters of interaction between APT\textsubscript{nAChR-α1} and the target (the fragment of nAChR α1), we performed Surface Plasmon Resonance (SPR) analysis, which revealed a dissociation constant (K\textsubscript{d}) of ~47 nM. Alexa488-APT\textsubscript{nAChR-α1} recognized the nAChR α1 positive cell lines and tissues successfully (Fig. 1b). However, Alexa488 conjugated APT did not bind to any sample.

**CONCLUSION**

We developed a nAChR α1-specific aptide (APT\textsubscript{nAChR-α1}) as a conjugation partner of therapeutic molecule to increase their lung retention time and bioavailability after pulmonary delivery. Results assured the feasibility of the current system.

**REFERENCES**


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