PEPTIDES 30 (2009) 343–350

available at www.sciencedirect.com

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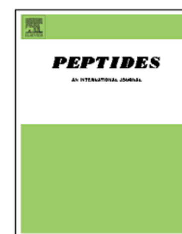

Identification of nose-to-brain homing peptide through phage display

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ARTICLE INFO

Article history:
Received 15 July 2008
Received in revised form
26 September 2008
Accepted 30 September 2008
Published on line 21 October 2008

Keywords:
Blood-brain barrier
Brain
Drug delivery
Intranasal
Phage display

ABSTRACT

Brain delivery of drug molecules through the nasal passage represents a viable approach for bypassing the blood-brain barrier (BBB) but remains a major challenge due to lack of efficient homing carriers. To screen for potential peptides with the ability to transport into the brain via the nasal passage, we applied a C7C phage peptide display library (Ph.D.-C7C) intranasally to anesthetized rats and recovered phage from the brain tissue 45 min after phage administration. After three rounds of panning, 10 positive phage clones were selected and sequenced. Clone7, which exhibited highest translocation efficiency, was chosen for further studies. After nasal administration, Clone7 entered the brain within 30 min and exhibited translocation efficiency about 50-fold higher than a random phage. A 11-amino acid synthetic peptide derived from the displayed sequence of Clone7 (ACTTPHAWLCG) efficiently inhibited the nasal-brain translocation of Clone7. Both phage recovery results and fluorescent microscopy images revealed the presence of many more Clone7 phage in the brain than in the liver, kidney and other internal organs after the nasal administration, suggesting that Clone7 bypassed the BBB and entered brain directly. Furthermore, both Clone7 and the ACTTPHAWLCG peptide were found to be heavily distributed along the olfactory nerve after the nasal administration, further suggesting a direct passage route into the brain via the olfactory region. These results demonstrated the feasibility of using the in vivo phage display approach for selecting peptides with the nose-to-brain homing capability and may have implications for the development of novel targeting carriers useful for brain delivery.

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1. Introduction

Many debilitating human diseases, such as Alzheimer's disease, stroke, brain tumor, head injury, depression, anxiety and other central nervous system (CNS) disorders, require efficient delivery of drugs into the brain [2,23] However, transporting drugs to the brain via the systemic circulation is a difficult task due to the presence of a tight blood-brain barrier (BBB) [2,6]. In fact, more than 98% of candidate CNS-targeting drugs have been halted during their mid-development as a result of poor permeability across the BBB. Thus, it is highly desirable to explore alternative route of delivery to transport drugs to the brain. One of the alternative methods for brain delivery is intranasal administration, which offers numerous advantages including rapid absorption, avoidance of hepatic first-pass metabolism, and patient convenience. Intranasal administration has been found to transport drugs preferentially to the brain via the olfactory pathway [14,16]. There is

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doi:10.1016/j.peptides.2008.09.026

also evidence indicating that certain viruses may enter the brain via the nasal mucosa [5]. Filamentous phage, known to reach the brain after intranasal administration, has been used as a carrier to deliver antibodies against amyloid plaques [10]. Borna disease virus may migrate intra-axonally from the neuroreceptors in the olfactory epithelium into the brain [17]. However, the brain homing efficiency of these viruses was quite low.

Peptide phage display is a selection technique in which a library of variants of a peptide is expressed on the outside of a phage virion, while the genetic material encoding each variant resides on the inside [11]. This creates a physical linkage between each variant peptide sequence and the DNA encoding it, which allows rapid partitioning based on binding affinity to a given target (antibodies, enzymes, cell-surface receptors, a material surface, etc.) by an in vitro selection process called panning [9,11]. In its simplest form, panning is carried out by incubating a library of phage-displayed peptides with a plate (or bead) coated with the target, washing away the unbound phage, and eluting the specifically bound phage. The eluted phage is then amplified and taken through additional binding/amplification cycles to enrich the pool in favor of binding sequences. After three to four rounds, individual clones exhibiting high affinity to the target may be obtained. An extension of this technique is in vivo phage display, which entails the injection of a peptide display phage library into the bloodstream of an animal and subsequent isolation and identification of phage that have the ability to home to a particular organ or tissue [3,19,20]. Other applications of the in vivo phage display include the identification of peptide sequences that facilitated the transport of phage across the gastrointestinal mucosal barrier [9] and the peptide sequence with the ability to overcome the skin barrier and deliver protein drugs transdermally [8]. These studies prompted us to ask whether in vivo phage display could be used to identify peptides with enhanced nose-to-brain homing capability. In the present paper we demonstrated the feasibility of this approach and showed that one of the selected phage (Clone7) could home preferentially to the brain along the olfactory pathway following nasal administration.

2. Materials and methods

2.1. *Materials and animals*

Ph.D.-C7C, phage library and mouse anti-M13 antibody were purchased from New England Biolabs (Beverly, MA, USA). Cy3 labeled anti-mouse IgG was purchased from Boster (Wuhan, China). Adult male Wistar rats (180–220 g) were obtained from the Medical Experimental Animal Center of Anhui Medical University. Peptide ACTTPHAWLCG (termed NB-7, the flanking A and G were derived from the M13 coat protein and were included to make the peptide more stable) was synthesized by GL Biochem Ltd (Shanghai, China), using standard solid-phase FMOC method with automatic peptide synthesizer (CS Bio) and purified to >95% by high-performance liquid chromatography (HPLC).

2.2. *Phage display*

In vivo phage selection was essentially performed as described [8], with a few modifications. The male Wistar rats were anesthetized with 20% solution of urethane (5 ml/kg). Rats were placed dorsally with heads positioned to maximize residency of exogenous substances on the olfactory epithelium 10^{11} plaque forming units (pfu) of Ph.D.-C7C phage library in 100 μl TBS (50 mM Tris–HCl, 150 mM NaCl, pH 7.5) were applied to the nasals of the rat. One hour after the phage administration, the rat was perfused through the heart with 100 ml of 0.01 M PBS (containing 0.5% Tween-20, pH 7.0). The cerebrum was withdrawn, weighed and homogenized with 1 ml Glycine buffer (0.2 M, pH 2.2). After centrifugation for 10 min (10,000 rpm), the supernatant was mixed with 0.5 ml of rapidly growing *Escherichia coli* ER 2738. After 30 min incubation, the recovered phages were amplified in 20 ml of Luria-Bertani (LB) medium for 6 h. Amplified phages were resuspended in TBS and used for the next round of in vivo selection. After three rounds of panning, enriched phages recovered from the third round were plated out on LB plates containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and IPTG (isopropyl-β-D-thiogalactoside). Ten blue plaques were randomly picked and subjected to DNA sequencing. With DNA Automatic Sequencer (ABI3730, Amersham Biosciences, Foster City, CA). The homologies of peptide sequence were multi-aligned by Clustal X [21].

2.3. *Peptide competition*

Phage homing assay was performed in the presence of increasing concentrations of synthetic peptide NB-7. Different amounts of peptide NB-7 were mixed with Clone7 phage and then applied to rat nose. The inhibitory effect of added peptide was quantified by evaluating phage titer as defined by the method described above.

2.4. *Immunohistochemistry*

To verify the specificity of Clone7 phage directly to brain, immunohistochemical analysis of Clone7 phage and random phage translocation was performed as described [18,19]. Clone7 or random phage or PBS was respectively given to three group rats as above. After 45 min, rats were exsanguinated and perfused through the heart with 50 ml 4% paraformaldehyde. After a further fixation step (24 h in 4% paraformaldehyde), cerebrum samples were immersed in 4.5% sucrose for 24 h and then dehydrated in 30% sucrose till deposition. Frozen sections were subject to immunohistochemistry, performed on 10 μm freezing sections blocked with 0.1% BSA for 1 h and mouse anti-M13 (1:100) was added for an addition 2 h at 37 °C. The sections were then washed three times for 5 min each in PBS and reaction with the secondary antibody (Cy3-labeled goat anti-mouse IgG, 1:500, 1 h, 37 °C). Finally, the cerebrum sections were washed three times in PBS and observed with OLYMPUS IX-70 microscope. Clone7 distributions in different tissues were observed.

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