Filamentous Bacterial Viruses Break Down Amyloid Plaques in an Animal Model of Alzheimer's Disease – A Novel Therapeutic Avenue

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The current dominant theory of Alzheimer's disease (AD) etiology and pathogenesis is related to the amyloid cascade hypothesis which states that overproduction of amyloid-beta-peptide (AβP), or failure to clear this peptide, leads to Alzheimer's disease primarily through amyloid deposition, presumed to be involved in neurofibrillary tangles formation [1]. Amyloid-β (Aβ) plaque formation, one of the main hallmarks of Alzheimer's disease, is a complex kinetic and thermodynamic process [2]. The dependence of AβP polymerization on peptide-peptide interactions to form a β-pleated sheet fibril, and the stimulatory influence of other proteins on the reaction suggest that amyloid formation can be modulated. Here we describe recent data on the use of filamentous phage as a delivery vector of anti-AβP antibodies which interfere with amyloid plaque formation [3], as well as novel therapeutics for disaggregation of amyloid plaques, towards a better alternative to existing attempts to treat AD.

Keywords: Filamentous phages, brain delivery vector, Alzheimer's disease, amyloid plaques

Bacteriophages can be found almost everywhere: from the ocean depths to hot springs, and can be isolated from soil and water as well as from the human or animal body (e.g. via saliva, feces, skin). Bacteriophages are known to be very common in the gastrointestinal tract and together with their bacterial hosts are an important component of gut flora [4,5]. All these facts reveal that mammalian organisms are very frequently exposed to interactions with bacteriophages and that these natural contacts are not incidental, but rather constant and intensive.

Classic phage therapy with lytic phages, as an alternative to antibiotics, was developed for clinical use by research groups in Europe and the USA, and has been extensively used in the former Soviet Union.

Unlike lytic phages which destroy the host cells, filamentous phages, such as M13, f1 or fd, did not affect the cells. They are well understood at both structural and genetic levels. They are bacterial viruses that consist of a circular single-stranded DNA (ssDNA) molecule, encapsulated in a protein envelope forming a rod-shaped cylindrical structure [6]. Two of the coat proteins – the major protein encoded by gene VIII (variously named pVIII) and the minor protein encoded by gene III – have surface-exposed N-terminal domains that tolerate foreign peptide inserts (see Figure 1). Protein pIII and the major coat protein pVIII have been used for peptide/protein phage display [7], thus making filamentous phage a valuable tool in many biological applications. For example, phage libraries were injected into mice and humans in attempting to identify antibodies or peptides that bind specifically to tissues of interest [8,9]. The expression of libraries of antibody fragments may therefore provide a method of by-passing hybridoma technology in generating monoclonal antibodies that home into a desired target [10].

Passive immunization of Tg mice model of Alzheimer's disease with phage-ScFv against β-amyloid peptide

Intranasal delivery provides a practical, non-invasive method for delivering therapeutic agents to the brain because of the unique anatomic connection provided by the olfactory and trigeminal nerves. Intranasal administration was chosen as a direct delivery route of vectors to the CNS via the olfactory neuron system or by close neuron tissue. Olfactory receptor neurons are bipolar cells that reside in the epithelial lining of the nose, high in the nasal cavity. Their axons traverse the cribriform plate and project to the first synapse of the olfactory pathway in the olfactory bulb of the brain. They seem to form a highway by which viruses or other transported substances may gain access to the CNS. The olfactory and trigeminal nerves connect the nasal mucosa and the CNS, allowing them to dectect odors and other chemical stimuli [11,12]. Intranasally administered drugs reach the parenchymal tissues of the brain and spinal cord and/or cerebrospinal fluid (CSF) within minutes using an extracellular route through perineural channels [12-14]. .

In addition to bypassing the BBB, the advantages of intranasal delivery include rapid delivery to the CNS, avoidance of hepatic first-pass drug metabolism, and elimination of the need for systemic delivery, thereby reducing unwanted systemic side effects. Intranasal delivery also provides painless and convenient self-administration by patients, features that encourage its use for delivering therapeutic agents into the CNS.

Filamentous bacteriophage proved to be an efficient and non-toxic viral delivery vector to the brain exhibiting penetration properties to the CNS [15] offering an obvious advantage over other mammalian vectors. The bacteriophage lacks the ability to infect mammalian cells, unless designed to do so. Due to its linear structure, the filamentous phage is highly permeable to different kinds of membranes and, following the olfactory tract, it may directly target affected sites in the brain.

The ability of anti-β-amyloid single-chain (ScFv) phage to dissolve Aβ plaques *in vivo* was demonstrated by repeated intranasal administration to hAPP transgenic mice [16,17].

The experiments were performed on transgenic mice carrying the hAPP gene with both London (717) and Swedish (670/671) mutations. The nine to ten months-old Tg mice were treated with ScFv phage. Administrations of 10 µl containing 10^{11} phages per mouse took place every 3 weeks for a total period of 6 months. Following passive immunizations protocol, the mice were subjected to training for the Morris Water Maze test for 4 days. The cognitive average of the treated animals was found to be close to that of the non-transgenic animals, indicating a healthy pattern of learning and memorizing of the new information [16,17].

Intranasal treatment for six months with phage anti-AβP ScFv of transgenic mice over-expressing hAPP resulted in reduction of plaque load and considerable reduction of brain inflammation. Thioflavin-S staining of mice-brain sections showed that plaque load in the treated mice was an average 50% of the plaque load in the control Tg mice [16,17].

These phages, engineered to display anti-Aβ antibodies, were delivered via intranasal administration into brains of transgenic mice and co-localized with Aβ plaques in a short time [15]. These findings are consistent with the extraneuronal pathway that has been proposed for transport of therapeutic agents from the nasal cavity into the brain [11-14]. This pathway occurs along the olfactory and trigeminal sensory nerons and likely involves extracellular bulk flow along perineuronal and/or perivascular channels, which delivers drug directly to the brain parenchyma, spinal cord, and perhaps the CSF. Thorne et al. reported that insulin-like growth factor-1 is rapidly transported into the brain and upper spinal cord within 30 min of intranasal administration via the extraneuronal pathway [12]. Delivery along the extraneuronal pathway is not receptor-mediated and requires only minutes for the drug to reach the brain [14].

This is the first demonstration that filamentous bacteriophage exhibits penetration properties to the CNS while preserving both the inert properties of the vector and the ability to carry foreign molecules. We showed a direct correlation between the number of applications and the amount of phage detected in the brain in both regions. The linear structure of the phage is suggested to confer penetration properties via various membranes. No evidence was shown of spread of filamentous phage to other brain sections, strongly emphasizing the olfactory tract as the most probable path in this case. In a control experiment, we performed intranasal administration of chloroform treated spheroid phages to the mice under the same experimental conditions and no presence of phages was detected [18].

Several hypotheses may be considered regarding the disappearance of the filamentous phage from the brain without inducing any toxic effect, as well as the long life-span of challenged animals [16,17]. As in other reported cases immune mechanisms may be involved that activate scavenger cells as microglia [19,20].

In experiments previously reported [8,9] filamentous phages were intravenously injected into mice and were subsequently rescued from the different organs, showing that their integrity was not affected during membrane penetration to the organs. They were genetically engineered to display both antigen and/or antibody and were used in different biological systems to present foreign proteins on their surfaces [10,21-23].

Filamentous phage binds and disaggregates amyloid plaques towards a new AD treatment

Recent data indicate that the filamentous phage not only stabilizes antibody fragments and enables their penetration into the brain but also exhibits anti-aggregating properties, inhibiting amyloid formation and dissolving already-formed aggregates. Here, we demonstrate the modulating effect of filamentous phage M13 on amyloid-β peptide aggregation and its ability to dissolve preformed aggregates. By modifying the phage's linear structure and rendering it spherical, we abolished its disaggregating as well as penetrating abilities [18].

We demonstrate that the linear structure of filamentous phage (a nanotubular appearance of 900 nm long and 7 nm diameter) dissolves β-amyloid plaques. *In vitro* anti-aggregating properties of filamentous phage on dissolving and/or preventing Aβ fibril formation were followed by

thioflavin (ThT) experiments. A decline of 26% in AβP aggregation was observed when the peptide was incubated in the presence of filamentous phage while addition of phages to preaggregated Aβ resulted in 45% reduction in amyloid fibrils. Experiments conducted in mice models of Alzheimer's disease showed therapeutic benefits of phage treatment. Mice were treated intranasally with filamentous phage for 12 months. During this period, cognitive tests were conducted to follow mice memory and olfaction functions. Phages administered intranasally to the mice improved cognitive and olfactory functions, protected neuronal degeneration, reduced brain inflammation and significantly decreased senile plaque load. Phages were eliminated from the brain and secreted from the body in urine and feces. No adverse effects were shown in peripheral organs: kidneys, liver, lungs and spleen biology was normal. The proposed new approach of intranasal application of phage presents several important advantages. First, it provides a rapid delivery route into the central nervous system (CNS) due to the unique connection between the nose and brain. A second advantage of using intranasal application is that the olfactory system, which was shown to be one of the first brain parts affected by Alzheimer;s disease, is targeted first. Moreover, phage therapy may overcome some of the drawbacks of β-amyloid immunotherapy, such as hemorrhages and inflammation.

Many studies are being conducted to prove the safety and efficacy of filamentous phage as well as the pharmacokinetics of phage particles [24-26]. Krag DN. *et al*. [25] evaluated the toxicity of repeated administrations of phage libraries to mice. The half-life of phage in plasma (in terms of recovered infectious particles) was calculated to be 3.6 h. After 72 h, phage was cleared from the blood, mainly through hepatic and renal excretion. This study demonstrated that injection of phages using a variety of regimens resulted in minimal toxicity.

Conclusions

Genetically engineered filamentous bacteriophage proved to be an efficient and non-toxic viral delivery vector of antibodies to the brain. Following the olfactory tract it may directly target affected sites in the brain, offering an obvious advantage over other mammalian vectors, as well as being an efficient immunocarrier for raising antibodies against AβP. Moreover, filamentous phage interferes with the Aβ aggregation process and dissolves existing Aβ fibrils by themselves.

The therapeutic potential of phages in AD, as well as in other amyloidogenic diseases, stems from their unprecedented ability to access the CNS, their ability to induce a potent anti-aggregating effect, and from their lack of a natural tropism for mammalian cells, resulting in no adverse effects.

This is the first attempt to use filamentous phage as a new and versatile therapeutic tool for treatment of a mouse model of Alzheimer's disease.

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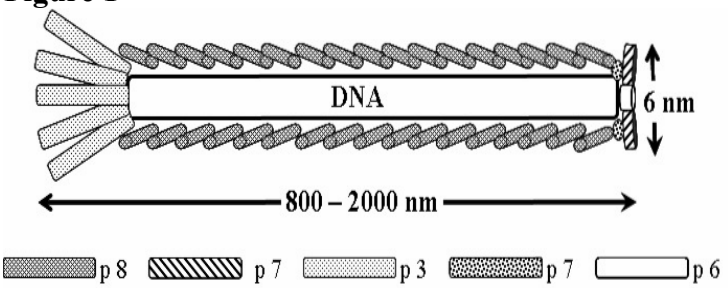
Figure 1



Schematic illustration of filamentous phage

The particle has diameter of 6-7 nm and length of ~ 800-2000 nm. The major coat protein, pVIII, is shown as small cylinders, as well as other minor proteins. Protein III can display scFv, while protein VIII can display small peptides, such as EFRH, without interfering with the life cycle of the phage.