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MPHAGES AND THE BLOOD-BRAIN BARRIER: A REVIEW

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ABSTRACT

The blood-brain barrier (BBB) is a protective barrier that prevents most substances from entering the brain from the bloodstream, including bacteria and viruses. Unfortunately, this restriction also applies to 99.9 % of therapeutics, posing significant challenges in the treatment of brain diseases. Overcoming this barrier is critical for effective treatment of neurological disorders. Several drug delivery systems are being developed to improve transport of therapeutic agent across the BBB. For example, nanoparticles (liposomes, polymeric nanoparticles, dendrimers), nanocarriers (micelles, nanogels), protein-based delivery (penetrating peptides, exosomes), focused ultrasound, and most recently, a filamentous phage based nanocarriers. Filamentous bacteriophages are viruses that infect bacteria and are not designed to infect eukaryotic cells. Recent evidence suggests that filamentous bacteriophages, such as M13, can cross BBB and enter the central nervous system (CNS). Researchers have been investigating the potential use of M13 as drug carriers, including the delivery of therapeutic agents to the brain. This entails modifying the bacteriophages to carry payloads such as drugs and using them as a delivery system. The BBB's complexity and the potential risks associated with changing it necessitate careful consideration in the development of such strategies. M13 nanocarrier development is ongoing, and advancements may lead to new therapeutic options for treating infections in the CNS. However, it is important to note that this field is still in its infancy, and more research is needed to assess the feasibility and safety of using modified bacteriophages to cross the BBB. This brief review attempts to compile current research on the potential use of bacteriophages for drug transport across the BBB.

Key words: bacteriophages; blood brain barrier; central nervous system; drug delivery systems; filamentous phages

THE BLOOD BRAIN BARRIER AND THERAPEUTICS

The BBB is formed by human brain microvascular endothelial cells (hBMECs) that line the cerebral microvessel from the luminal side. The endothelial cells are covered by pericytes and supported by glial cells (astrocytes) and neurons, all of which work together to maintain normal brain

function and play an important role in barrier function and immune responses. Semipermeable BBB is distinguished by the presence of highly specialized interendothelial tight junctions. Some of the characteristics that distinguish BBB from other barriers are as follows: i) the presence of cell-cell tight junctions; (ii) the absence of fenestrae and a lower level of fluid-phase endocytosis; (iii) asymmetry in enzyme localization [41]. In the brain, homeostasis is more important than anywhere else in the body. The BBB protects the brain from the frequent ion fluctuations that occur in plasma. Small lipid-soluble molecules like carbon dioxide or ethanol can easily pass through the barrier thanks to the lipid membranes. The harmful molecules are swiftly expelled from the BBB via efflux transport proteins (like P-glycoprotein-1). In the absence of a specialized carrier-mediated transport system, water soluble molecules and macromolecules are unable to cross the BBB [33].

Treatment for brain diseases is notoriously difficult. The blood-brain barrier, which surrounds the brain parenchyma and is nearly impenetrable, is the underlying cause of the difficulty in brain treatment [22]. The BBB is the most intact of all cell barriers due to the compact layout of endothelial cells and intercellular tight junctions, while efflux mechanisms prevent unwanted molecules from entering the brain [39]. Furthermore, as illustrated in the cartoon, each extremely narrow interendothelial space is practically lined with pericytes and supported by astrocyte end feet (Figure 1). The presence of BBB was discovered in the nineteenth century by Paul Ehrlich, who observed the inability of "intravital tracer dye" to pass through the brains of mice [12].

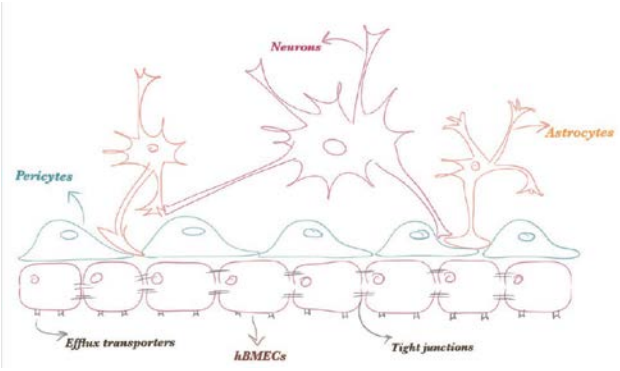

Fig. 1. A cartoon illustrating the blood brain barrier. The BBB is a specialized system that protects the brain from toxic substances in the blood while also filtering harmful compounds from the brain back into the bloodstream.

Despite the fact that there are over 600 therapeutics on the market today, the vast majority of brain diseases remain incurable [44]. For more than two decades, scientists have been researching various biomaterials, synthetic transport systems, nanocarriers, and viruses in order to transport a drug payload from blood to the CNS. Bacteriophages are one of the tools being explored as a carrier. Bacteriophages are the most numerous biological entities and have been demonstrated to be effective against antibiotic-resistant bacteria. They are highly host specific, typically infecting only one type of bacteria. A phage particle is composed of a single nucleic acid, either DNA or RNA and a protein capsid that protects the genetic material [32]. Bacteriophages cannot infect eukaryotic cells, but they can pass through epithelial cell layers and spread throughout the body, including the blood, lymph, kidney, spleen and even the brain [34]. Because they enter the body through food, their concentration in the gut remains high [34]. Various types of the bacteriophages are illustrated in Figure 2.

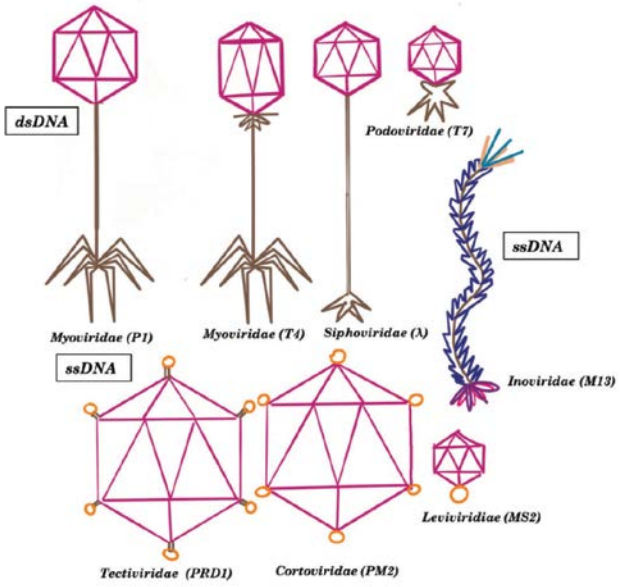

Fig. 2. Classification of bacteriophages based on morphology and properties of DNA

Filamentous bacteriophages

Filamentous bacteriophages (genus *Inovirus*) resemble a flexible rod and contain circular single-stranded DNA that codes for approximately ten genes. They attack Gram-negative bacteria specifically, and infection is distinguished by the unique strategy of virion morphogenesis. F-specific filamentous phages (Ff) are the most well-stud-

ied of the various filamentous phages. Filamentous phages M13, fd, and f1 are members of the *Inoviridae* family, which includes several subfamilies with distinct structures and lifecycles [9, 16]. Their "body" is comprised of multiple proteins. The backbone is formed by a major coat protein (or pVIII protein), while minor coat proteins are found on both ends, created by pIII and pVI proteins on one end and pVII and pIX on the other [21]. Minor coat proteins are required for interaction with the F pilus of the target bacteria [25]. A structure of filamentous phage is illustrated in Figure 3.

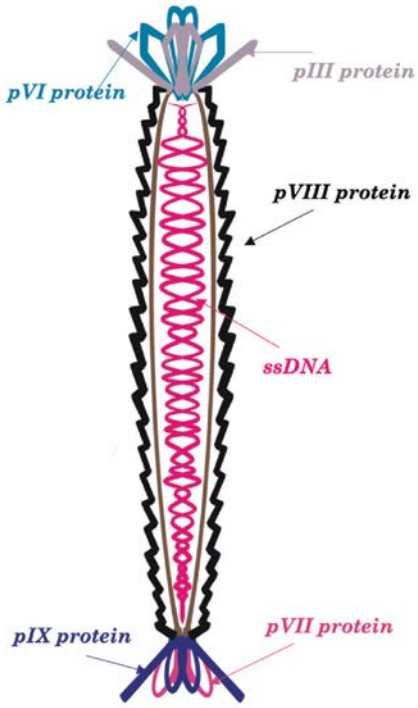

Fig. 3. A filamentous bacteriophage

Filamentous phages can be used as a drug carrier into the BBB when administered intranasally or via convection-enhanced delivery (CED) [42]. The use of filamentous phage as a carrier appears to be a more acceptable non-invasive approach among the many strategies being tested to transport drugs across the BBB. While invasive techniques rely on harsh methods such as chemical opening of the BBB, ultrasound disruption of the BBB, or even intracerebral infusions [3], non-invasive methods primarily rely on nanotechnology-based carrier systems (nanocarriers). Several effective nanocarriers are being developed, including carbon dots, carbon nanotubes, dendrimers, polymeric nanoparticles, and viral vectors (including filamentous phages), liposomes, and micelles [1, 45]. Thermodynamic stability, biocompatibility, homogeneity, high carrying capacity, self-assembly, scalability, and low toxicity are all advantages of phage-based carriers over other nanoparticles [43]. In addition to many other peptides, they can be used to transport nucleic acids [23]. BBB homing peptides (peptides that aid in the transportation of large molecules across BBB), organ/cell-specific peptides or pathogen-specific peptides can be selected using a phage display technology using filamentous phages [21]. The display technology was discovered by Nobel prize winner George P. Smith in 1985, which has enabled the discovery of plethora of proteins, peptides and antibodies for diagnostic and therapeutic purposes [37].

BBB homing: Transportation from blood to brain

Several BBB homing peptides have been discovered to treat various brain tumours and CNS infections. Choi-Fong and colleagues identified BTP-7, a targeting peptide for the treatment of high-grade gliomas [8]. The majority of the studies used phage display to identify BBB homing peptides. Commercially available combinatorial phage libraries (Ph.D.-12, Ph.D.-7, and Ph.D.-C7C) from New England Biolabs are being used frequently in display experiments [28]. For example, the C7C peptide with the sequence A**C**TTPHAWL**C**G was developed using the combinatorial phage display technique and was able to cross the BBB within 45 minutes of administration [42]. The majority of homing peptides discovered to date are taken up by brain microvascular endothelial cells via active transport, such as receptor-mediated endocytosis [30].

There is some evidence that filamentous phages can cross the BBB [30, 34, 43, 46]. Thus, the phage body can be used as a vehicle to transport therapeutics into the brain. There are several proposed mechanisms for phage entry across the epithelial cell layer. They include leaky gut syndrome, in which phages can pass through the epithelial cell layer due to cellular damage, and the "Trojan horse" mechanism, in which bacteriophages can enter by being engulfed by bacteria [2, 20]. Another mechanism under consideration is the free uptake of phage particles by eukaryotic cells via endocytosis [2, 6, 18]. Several experiments were carried out to assess phage transcytosis through epithelial cell layers. T4 bacteriophage transcytosis was tested in various cell types representing different organs for this purpose. They were: the gut (T84 and

CaCo2), the lung (A549), the liver (Huh7), and the brain (hBMEc). T4 bacteriophages showed transcytosis in both directions, but preferentially from apical to basal [34]. It was hypothesized that phages transit through the Golgi apparatus before being exocytosed [34].

One of the first discoveries of the phage passage through the BBB, was in 1943 when Dubos et al. [11] [demonstrated that anti-Shiga bacteriophages were accumulated and replicated within the brains of mice infected with *Shigella dysenteriae*. Keller and Engley [24] conducted another experiment in 1958 using bacteriophages selected against *Bacillus megatherium*. They found similar accumulation and persistence in the brains of mice after intraperitoneal injections for up to 6 hours. The question is whether naturally occurring bacteriophages in the brain aid in some function. They are thought to protect against meningitis caused by commensal bacteria [2]. Filamentous M13 bacteriophages were examined for presence in the brain, and one of the first studies on primate brains revealed that bacteriophages spread throughout the primate brain via axonal transport [27]. Many studies are currently underway to investigate the transport of phages or phage-displayed peptides through the brain in order to cure or prevent a variety of brain-related diseases. In addition, designing M13 filamentous bacteriophages capable of penetrating the BBB and targeting glioblastoma, a neuroectodermal tumour, was a significant step forward. M13 were created by reducing their genome size, which correlates with phage particle length. By using two distinct phages, one containing packaging components and the other containing only ssDNA with tunable length and a packaging signal, their length was reduced [40]. Phage therapy for brain diseases such as Alzheimer's and Parkinson's disease, as well as brain infections such as *Borrelia burgdorferi*, Flaviviruses, Listeria, and others, has received attention in the last decade [7, 14]. The M13 phage was discovered to be capable of disintegrating alpha-synuclein inside the brain of a mouse model [10]. The alpha-synuclein is considered one of the main factors that causes and accompanies Alzheimer's and Parkinson's disease. Ff phages can also cross the BBB and may be used in future therapeutic approaches [43]. Fd filamentous bacteriophages with cocaine-binding properties were also shown to block cocaine's psychoactive effects in the brain [5]. Phage based therapy was also developed against *Borrelia* infection. In order to treat borreliosis, induced native phage therapy was developed, which relies on phages that naturally coexist in the human body. Using this treatment, 77 percent of 26 Lyme disease syndrome patients were cured [19]. Phage display technology is also used to display specific peptides against viruses such as West Nile virus, SARS-Cov-2, Dengue virus, and Hepatitis C virus [4, 17, 38, 47]. Specific single-domain antibodies were designed to block domain III (DIII-$2^{V371-R388}$) of the envelope protein of the West Nile virus [17].

The mechanisms of the bacteriophage traversal through BBB is not yet fully known. One study suggests the crossing of phages through both routes, through vesicular and cytosolic compartments of the eukaryotic cell. During the transcytosis, they traffic through the Golgi apparatus via the endomembrane system [34]. There is evidence that filamentous bacteriophages have the ability to penetrate the central nervous system while retaining vector properties and the ability to carry foreign molecules [13]. It was discovered that the proline at position 213 of the surface protein pIII is critical for forming a hinge between two amino-terminal domains during interaction with the bacterial pili. The Gln212-Pro213 peptide bond undergoes subsequent isomerization from trans to cis conformation [31]. As a result, only a G3P or pIII peptide essential for interacting with host cells should be used as a homing peptide, rather than the entire phage. It was discovered that the pIII peptide alone was nearly as effective as the entire M13 phage in interacting with amyloid fibres in the brain [26]. G3P has three domains (N1, N2, and C), each of which performs a different mechanism of action [29]. The C domain is responsible for releasing a viral particle from the infected cell [36]. As a result, only domains responsible for penetrating cells and not passing out should be used as homing peptides.

Bacteriophages have several advantages as therapeutic agents against pathogens or as BBB homing agents. Specifically, 1) their high host specificity prevents them from having negative side effects on commensal microbiota, 2) their self-replication within chosen bacterial targets maintains their population at the specific site of infection, 3) they cease to replicate when the infection is eradicated, and 4 they are effective even against multi-resistant bacterial strains [15, 35].

CONCLUSIONS

The BBB is the most difficult barrier to overcome in the treatment of brain diseases because drug penetration is extremely limited. Bacteriophages are emerging as a promising tool in the search for an efficient carrier to transport therapeutic molecules from the blood to the brain, owing to their high scalability, flexibility, and ability to cross various physiological barriers. A truncated model of phage or phage proteins (like pIII) alone could be investigated further as carrier systems. For example, an antimicrobial peptide fused with pIII may be able to cross the BBB while retaining antibacterial activity in the brain parenchyma. Bacteriophages and their proteins open up new avenues for the development of CNS drug delivery systems. It is important to note that the use of bacteriophages in medical treatments is an evolving field, and further studies are needed to fully understand their safety and efficacy, especially in the context of CNS.

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