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Review

siRNA as a tool to improve the treatment of brain diseases: Mechanism, targets and delivery

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ABSTRACT

As the population ages, brain pathologies such as neurodegenerative diseases and brain cancer increase their incidence, being the need to find successful treatments of upmost importance. Drug delivery to the central nervous system (CNS) is required in order to reach diseases causes and treat them. However, biological barriers, mainly blood–brain barrier (BBB), are the key obstacles that prevent the effectiveness of possible treatments due to their ability to strongly limit the perfusion of compounds into the brain. Over the past decades, new approaches towards overcoming BBB and its efflux transporters had been proposed. One of these approaches here reviewed is through small interfering RNA (siRNA), which is capable to specifically target one gene and silence it in a post-transcriptional way. There are different possible functional proteins at the BBB, as the ones responsible for transport or just for its tightness, which could be a siRNA target. As important as the effective silence is the way to delivery siRNA to its anatomical site of action. This is where nanotechnology-based systems may help, by protecting siRNA circulation and providing cell/tissue-targeting and intracellular siRNA delivery. After an initial overview on incidence of brain diseases and basic features of the CNS, BBB and its efflux pumps, this review focuses on recent strategies to reach brain based on siRNA, and how to specifically target these approaches in order to treat brain diseases.

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1. Introduction

The World Health Organization has indicated that central nervous system (CNS) disorders are the major medical challenge of the 21st Century (ResearchAndMarkets, 2007). Disorders of the CNS are numerous, diverse, frequently severe, and affect a large portion of the world population. These diseases can debilitate conditions that significantly affect the morbidity and mortality of modern society. Neurodegenerative diseases including Alzheimer's diseases (AD), Parkinson's diseases (PD) and amyotrophic lateral sclerosis – which symptoms are related to loss of movement, memory, and dementia due to the gradual loss of neurons – are constantly and rapidly increasing as population ages. As well, brain tumours constitute a severe and unsolved clinical condition and are a common cause of cancer-related death.

Longer life expectancy should be followed by better quality of life, however, current therapies to CNS disorders (which are mainly incident on old-age population) do not positively correspond to their expectations (Bhaskar et al., 2010). Neurodegenerative diseases, deeply associated with ageing, are usually linked to a loss of brain and spinal cord cells. As examples, in AD and PD the neuronal damage occur due to abnormal protein processing and accumulation, which results in gradual cognitive and motor deterioration (Gilmore et al., 2008).

According to Brain Tumour Research website statistics (Farm, 2013), brain tumours kill more children and adults under the age of 40 than any other cancer, and only 18.8% of those diagnosed with brain tumour survive beyond five years, compared to 50% average survival prognostic for all cancers. Moreover, the incidence of brain metastases has increased over the last decade mainly due to improved treatment of primary peripheral cancers resulting in increased patient survival, as well as due to the development of newer tools to image and diagnostic tumours of the CNS (Agarwal et al., 2011). Among the different ways to treat cancer, such as surgery and radiotherapy (which uses high-energy particles or waves to destroy or damage cancer cells, that arise the possibility to damage also normal cells, reason why this treatment must be carefully planned to minimize side effects), tumour therapy is usually based on the interplay between chemotherapeutic and antiangiogenic agents (Murthy, 2007). In general, treatment of many ageing disorders and tumours require drugs acting on the CNS, highlighting the need and importance to reach CNS on a therapeutic concentration. Simultaneously, the field of nanomedicine is rapidly expanding and promises revolutionary advances to the diagnosis and treatment of devastating human diseases (Gilmore et al., 2008).

2. Obstacles to brain diseases treatment

Drug delivery to the CNS represents a challenge in developing effective treatments for neurodegenerative diseases and brain tumours due to the unique and complicated environment imposed by the CNS itself. There are protective barriers which restrict the passage of foreign substances into the brain, namely the blood–brain barrier (BBB), the blood cerebrospinal fluid barrier (BCSF), and other specialized CNS barriers as the arachnoid barrier (Abbott et al., 2010; Bhaskar et al., 2010). Therefore, an important part of this CNS challenge is overcoming the natural tendency of the BBB to block drug transport. This barrier, a tightly packed layer of endothelial cells surrounding the brain (Bhaskar et al., 2010), is designed to protect and prevent high-molecular weight molecules in blood from entering the brain by filter harmful compounds from the brain back to the bloodstream. As the BBB cannot recognize many therapeutic compounds, high doses must be administered to have a drug therapeutic concentration at the brain, with increased risks of adverse side effects (Murthy, 2007).

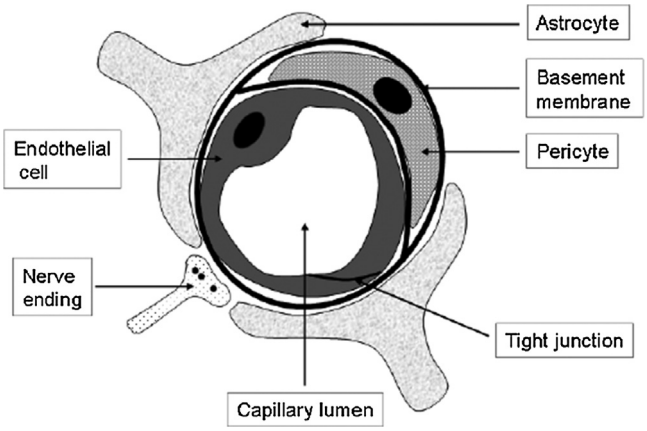

Fig. 1. BBB cellular structure (Wilhelm et al., 2011).

Due to the difficulty of physically active molecules overcome BBB and reach CNS, it becomes crucial to understand the structural composition as well as how the factors that regulate permeability of the substances across the BBB act. BBB is constituted by the brain endothelial cells which form the cerebral microvascular endothelium. The cerebral microvascular endothelium, together with astrocytes, pericytes, neurons, and the extracellular matrix, constitute a "neurovascular unit" that is essential for the health and function of the CNS (see Fig. 1). Pericytes play an important role on the integration of endothelial cells and astrocytes functions at the neurovascular unit (Armulik et al., 2010; Fisher, 2009); astrocytes are crucial on the induction of BBB functions; and neurons on BBB cerebral flow and vessel dynamics (Cardoso et al., 2010; Choi and Kim, 2008; Weiss et al., 2009). Several molecular and receptor structures are present on the surface of the endothelial cells, able to mediate the transport of solutes and other substances including drugs in and out of the brain. BBB is also responsible for leucocyte migration and maintenance of brain microenvironment homeostasis, which is crucial for neuronal activity and proper functioning of CNS. The transport of solutes and other substances across BBB is also dependent on tight junctions (TJs) between adjacent endothelial cells, adherent junctions (AJs) and metabolic barriers (enzymes, diverse transport systems). Besides TJs, as special characteristics of BBB that limit drug uptake, there is also a lack of fenestrations and a low endogenous pinocytotic activity.

Next to the BBB, BCSF is the second important feature of the CNS, formed by the epithelial cells of the choroid plexus. BCSF mainly regulates the exchange of molecules between the blood and CSF, controlling the penetration within the interstitial fluid of the brain parenchyma (Bhaskar et al., 2010; Gilmore et al., 2008; Mahringer et al., 2011). Moreover, another interface, the avascular arachnoid epithelium, has a relatively small surface that is the main reason why it is not a significant surface for exchange between blood and CNS (Abbott et al., 2010). Some other CNS barriers, like blood tumour barrier and blood retina barrier, may also play a role in drug transport (Bhaskar et al., 2010).

Concerning these limitations, some current strategies used for drug delivery to the brain include invasive delivery, temporary disruption of the BBB, as well as the use of specific drug delivery systems. While direct injection can be an effective invasive modality for local delivery in some cases (*e.g.*, in some tumours), it is not efficient for brain metastasis or neurodegenerative diseases, which require therapeutic agents to be widely spread in the brain. Reversible opening of the BBB, by an osmotic or chemical method as well as ultrasound techniques, allows therapeutic agents to enter the brain. However, this approach can also result in significant damage to the brain (Gilmore et al., 2008), with partial irreversibility of