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shRNA and siRNA delivery to the brain ☆

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Abstract

The limiting factor in in vivo RNA interference (RNAi) is delivery. Drug delivery methods that are effective in cell culture may not be practical in vivo for intravenous RNAi applications. Nucleic acid drugs are highly charged and do not cross cell membranes by free diffusion. Therefore, the in vivo delivery of RNAi therapeutics must use targeting technology that enables the RNAi therapeutic to traverse biological membrane barriers in vivo. For RNAi of the brain, the nucleic acid-based drug must first cross the brain capillary endothelial wall, which forms the blood–brain barrier (BBB) in vivo, and then traverses the brain cell plasma membrane. Similar to the delivery of non-viral gene therapies, plasmid DNA encoding for short hairpin RNA (shRNA) may be delivered to the brain following intravenous administration with pegylated immunoliposomes (PILs). The plasmid DNA is encapsulated in a 100 nm liposome, which is pegylated, and conjugated with receptor specific targeting monoclonal antibodies (MAb). Weekly, intravenous RNAi with PILs enables a 90% knockdown of the human epidermal growth factor receptor, which results in a 90% increase in survival time in mice with intra-cranial brain cancer. Similar to the delivery of antisense agents, short interfering RNAi (siRNA) duplexes can be delivered with the combined use of targeting MAb's and avidin–biotin technology. The siRNA is mono-biotinylated in parallel with the production of a conjugate of the targeting MAb and streptavidin. Intravenous RNAi requires the combined use of RNAi technology and a drug targeting technology that is effective in vivo.

Keywords: Blood–brain barrier; RNAi; Endothelium; Transferrin receptor; Insulin receptor; Monoclonal antibody

Contents

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1. Introduction

RNA interference (RNAi) is a new strategy for the development of antisense therapeutics that knock down gene expression post-transcriptionally [1]. RNAi mechanisms may involve either the degradation of target RNA, e.g. in the case of a short interfering RNA (siRNA), or cause translation arrest of the target RNA, e.g. in the case of micro RNA (miRNA). There are two types of RNAi-based therapeutics: DNA-based RNAi and RNA-based RNAi. In DNA-based RNAi, a plasmid DNA encodes for a short hairpin RNA (shRNA). In RNA-based RNAi, a siRNA duplex is chemically synthesized without a DNA intermediate.

Drug delivery is the rate-limiting step in the translation of RNAi-based therapeutics from cell culture to in vivo therapeutics in animals and humans. Many delivery methodologies that prove effective in cell culture may be difficult to implement in vivo, because biological membrane barriers exist in vivo that are absent in a Petri dish. Consequently, the examples of therapeutic effects in vivo with "intravenous RNAi" are few compared to the biological effects of RNAi demonstrated in cell culture. The goal of intravenous RNAi is to inject the RNAi therapeutic intravenously and obtain knock down of the target gene in the target organ without toxicity.

The role of drug delivery in the development of in vivo intravenous RNAi may be summarized as follows:

- The problems of shRNA delivery in vivo recapitulate the problems of gene delivery in vivo.
- The problems of siRNA delivery in vivo recapitulate the problems of antisense delivery in vivo.

The size of the RNAi delivery problem is illustrated by consideration of the development of antisense and gene therapeutics, both of which are >20 years in development. Presently, there are no FDA approved gene therapies, and no FDA approved drugs that work via an antisense mechanism. Yet, both gene therapy and antisense drugs have powerful effects in cell culture. Neither gene therapy nor antisense drugs have been translated into FDA approved therapeutics, because of the rate-limiting role played by in vivo drug targeting of these highly polar, large molecular weight agents. The fields of either gene therapy or antisense failed to develop effective targeting technology that enables clinically significant therapeutic effects of these agents in vivo following intravenous administration.

Many of the present-day RNAi delivery methods simply recapitulate the delivery approaches used in the past for antisense and gene therapy. If the delivery technologies were largely ineffective in vivo for antisense delivery or gene delivery, then how can these delivery systems work for in vivo RNAi? In the absence of the development of new delivery technology for nucleic acid therapeutics, it is possible that RNAi therapeutics will follow the same development path as antisense and gene therapy.

The present chapter will review the use of molecular Trojan horses (MTH) to deliver non-viral plasmid DNA therapeutics, and antisense drugs to the brain, and other organs, in vivo following intravenous administration. The chapter will review how plasmid DNA delivery technology is adapted to the problem of the delivery of shRNA encoding plasmid DNA, and how the antisense delivery technology is adapted to the problem of siRNA delivery in vivo. However, MTHs, alone, cannot easily deliver these agents to brain and other organs in vivo. The MTHs must be combined with other formulation technologies, which enable an efficient, and metabolically stable attachment of the nucleic acid therapeutic to the MTH. Therefore, this chapter will also review the use of the Trojan horse liposome (THL) technology, also called the pegylated immunoliposome (PIL) technology, which combines plasmid DNA and MTHs in a formulation that enables transport of the DNA across biological barriers in vivo following intravenous administration. In the case of antisense, and siRNA, the chapter will review avidin–biotin (AB) technology, which combines the MTH and the antisense or siRNA in a formulation that is stable in vivo.

2. Overview of blood–brain barrier molecular Trojan horses

2.1. Blood–brain barrier receptor-mediated transport (RMT)

The blood–brain barrier (BBB) is formed by the brain capillary endothelial wall [2]. Epithelial-like, high resistance tight junctions cement brain capillary endothelial cells together, eliminating endothelial pores that exist in the capillaries perfusing non-brain tissues. Therefore, there is no para-cellular pathway of solute exchange between blood and brain. There is also minimal pinocytosis in brain capillary endothelial cells. Therefore, there is no significant trans-cellular pathway for free solute exchange between blood and brain. Consequently, there are only two mechanisms by which molecules in the blood may gain access to brain interstitial fluid: (a) lipid-mediated transport of lipid soluble small molecules with a molecular weight <400 Da; and (b) catalyzed transport [3]. Catalyzed transport includes carrier-mediated transport (CMT) for small molecules and receptor-mediated transport (RMT) for large molecules. The plasma membrane of brain capillary endothelial cells express various CMTs, such as the GLUT1 glucose transporter, the LAT1 large neutral amino acid transporter, the CAT1 cationic amino acid transporter, the MCT1 monocarboxylic acid transporter, and the CNT2 adenosine transporter [4]. In addition to catalyzed transport of endogenous small molecules via the BBB CMT systems, there is also RMT of endogenous large molecules across the BBB. The