International Congress Series 1277 (2005) 185–198

www.ics-elsevier.com

Diphtheria toxin receptor-targeted brain drug delivery

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Abstract. Brain drug delivery is limited by the blood–brain barrier (BBB). We have newly identified the membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF), which is also known as the diphtheria toxin receptor (DTR), as a well characterized internalizing transport receptor on the BBB for the targeting of drugs to the brain. This transport receptor has several unique advantages. It makes use of a non-toxic endogenous transport mechanism called receptor-mediated endocytosis, with proven cargo-carrying properties across the BBB (e.g., brain delivery (transcytosis) of large proteins and liposomes containing drugs); the receptor has no endogenous ligands and thus neither competition from endogenous ligands, nor blockade of transport to the brain of essential nutrients is to be expected; the membrane bound receptor is constitutively expressed on the BBB, neurons, and glial cells; receptor expression is highly amplified in disease conditions and thus allows for site-specific disease targeting; and the biological activity of the receptor can be modulated by a variety of pharmacologically active compounds (like heparin and proteinase inhibitors). Furthermore, the targeting technology makes use of a non-toxic mutant of diphtheria toxin (known as CRM197) as the receptor-specific carrier protein. This carrier protein has several unique advantages as well. It is a well characterized protein (i.e., known receptor binding domain, conjugation sites, manufacturing process), and it has already been successfully marketed for human use to millions of people in vaccination programs, and recently also in anti-cancer trials, with a proven carrier efficacy and excellent safety profile. We have been able to demonstrate proof-of-principle data in our cell culture model of the BBB, as well as in guinea pigs with this novel brain drug targeting technology, including: functional expression of DTR; safety of CRM197 carrier protein; transport efficacy of CRM197 carrier protein conjugated directly to horseradish peroxidase (HRP, serving as a 40 kDa 'model' protein drug); and specific in vivo brain uptake of DTR-targeted HRP. In conclusion, the

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doi:10.1016/j.ics.2005.02.022

DTR seems to be a human applicable, safe, and effective uptake receptor for the targeting of drugs to the brain.

Keywords: Blood–brain barrier; Heparin-binding epidermal growth factor; Diphtheria toxin receptor; Receptor-mediated endocytosis; Drug targeting

1. Introduction

Brain drug delivery is limited by the blood–brain barrier (BBB). Particularly, hydrophilic and large drugs (e.g., biopharmaceuticals) poorly pass the blood–brain barrier and generally do not reach the brain in sufficient concentrations to be effective. As a result, many high potential 'would be' central nervous system (CNS) drugs (especially biopharmaceuticals) are currently not available to the brain [1]. Unlike small molecules, large biopharmaceutical drugs are unlikely candidates for chemical modifications to enhance their permeability across the blood–brain barrier. Invasive and potentially harmful technologies to patients, like direct stereotactic injections, intrathecal infusions, and blood–brain barrier disruption, are now being evaluated in clinical settings. Because of the severe neurological consequences, these techniques are only allowed to be applied in selected life-threatening diseases. Moreover, these technologies are far from effective in delivering drugs throughout the whole brain. Therefore, there is an established need to improve the delivery of biopharmaceutical drugs to the brain.

Since almost every neuron is perfused by its own capillary, the most effective way of delivering biopharmaceutical drugs is achieved by targeting to endogenous transport receptors on these capillaries. In fact, the total length of capillaries in the human brain is impressive (~600 km) with a large surface area (~20 m^2) for effective exchange of drugs [2]. An intravenous (injectable) drug delivery technology for CNS-active biopharmaceutical drugs will allow for the (enhanced) treatment of many more brain disorders. In many preclinical studies, the insulin- or (melano)transferrin receptors have been successfully utilized for this purpose [2]. Currently, there are, however, no such brain drug delivery technologies on the market for human use. One reason for this might be that these technologies still involve potential safety hazards, like the obstruction of brain entry of essential compounds (like insulin or iron), or potentially dangerous interactions with endogenous substrates (as was published for melanotransferrin/p97 which seems to activate plasminogen in rats [3]). Therefore, there still exists a need for new, safe, and effective brain drug delivery technologies that can potentially be applied to humans.

In this paper, we describe the use of the receptor for diphtheria toxin (DTR), the membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF), as a new endogenous transport receptor for the delivery of drugs across the BBB. Specifically, we used CRM197, a non-toxic mutant protein of diphtheria toxin, as the receptor-specific carrier protein. CRM197 has already been used as a safe and effective carrier protein in human vaccines for a long time [4], as well as in recent anti-cancer trials [5]. This resulted in a large body of prior knowledge on the carrier protein, including its transport receptor and mechanism of action, receptor binding domain, conjugation- and manufacturing process, and kinetic- and safety profile both in animals