Advanced Drug Delivery Reviews 65 (2013) 1012–1019

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Structure and dynamics of drug carriers and their interaction with cellular receptors: Focus on serum transferrin ☆

Ashley N. Luck [1], Anne B. Mason *

Department of Biochemistry, University of Vermont, College of Medicine, 89 Beaumont Avenue, Burlington, VT 05405, USA

ARTICLE INFO

Article history:
Accepted 14 November 2012
Available online 23 November 2012

Keywords:
Transferrin
Transferrin receptor
Trojan horse
Chemotherapy

ABSTRACT

Highly proliferative cells have a dramatically increased need for iron which results in the expression of an increased number of transferrin receptors (TFR). This insight makes the transferrin receptor on these cells an excellent candidate for targeted therapeutics. In this regard, it is critical to understand at a molecular level exactly how the TFR interacts with its ligand, hTF. Understanding of the hTF/TFR pathway could, in theory, maximize the use of this system for development of more effective small molecules or toxin-conjugates to specifically target cancer cells. Many strategies have been attempted with the objective of utilizing the hTF/TFR system to deliver drugs; these include conjugation of a toxin or drug to hTF or direct targeting of the TFR by antibodies. To date, in spite of all of the effort, there is a conspicuous absence of any successful candidate drugs reaching the clinic. We suggest that a lack of quantitative data to determine the basic biochemical properties of the drug carrier and the effects of drug-conjugation on the hTF-TFR interaction may have contributed to the failure to realize the full potential of this system. This review provides some guidelines for developing a more quantitative approach for evaluation of current and future hTF-drug conjugates.

© 2012 Elsevier B.V. All rights reserved.

Contents

1. Introduction . . . 1013
2. hTF/TFR specificity . . . 1013
 2.1. Structure of the hTF/sTFR complex . . . 1013
  2.1.1. hTF N1-TFR motif . . . 1013
  2.1.2. hTF N2-TFR motif . . . 1014
  2.1.3. hTF C1-TFR motif . . . 1015
  2.1.4. Changes in the structure of the sTFR as a result of the binding of Fe_NhTF hTF . . . 1015
3. Targeting the hTF/TFR for chemotherapeutic treatments . . . 1015
 3.1. Issues to consider in targeting hTF/TFR . . . 1015
 3.2. Evaluation of TF conjugates . . . 1015
  3.2.1. Binding studies to provide relative binding affinities of conjugates versus native TF . . . 1017
  3.2.2. Experimental systems used in evaluation . . . 1017
  3.2.3. Recent innovations . . . 1017
4. Conclusion . . . 1018
Acknowledgments . . . 1018
References . . . 1018

Abbreviations: apoTF, serum transferrin that is iron free; BBB, blood brain barrier; EM, electron microscopy; Fe_ChTF, recombinant N-terminal hexa-His tagged non-glycosylated monoferric hTF that binds iron only in the C-lobe; Fe_NhTF, recombinant N-terminal hexa-His tagged non-glycosylated monoferric hTF that binds iron only in the N-lobe; Fe_2hTF, recombinant N-terminal hexa-His tagged non-glycosylated diferric hTF; HFE, the hemochromatosis protein; hTF, human serum transferrin; scFv, single chain antibody variable region; SPR, surface plasmon resonance; sTFR, glycosylated N-terminal hexa-His tagged soluble recombinant transferrin receptor (residues 121–760); ITC, isothermal titration calorimetry; TFR, transferrin receptor.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Delivery of biopharmaceuticals: Advanced analytical and biophysical methods".

* Corresponding author at: Department of Biochemistry, University of Vermont, Burlington, VT 05405, USA. Tel.: +1 802 656 0343; fax: +1 802 862 8229.
E-mail address: anne.mason@uvm.edu (A.B. Mason).
[1] Previously Ashley N. Steere, Current address: New England Biolabs, 240 County Road, Ipswich, MA 01938, USA.

0169-409X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.addr.2012.11.001

1. Introduction

Given the potential for detrimental effects due to the redox properties of the Fe^{2+}/Fe^{3+} pair, once it enters the body iron that is acquired from the diet is tightly regulated. Specifically, iron, in the form of Fe^{3+}, is carefully shielded from exposure to the aqueous environment of the blood by sequestration within each cleft of the iron binding and transport protein, human serum transferrin (hTF). An ~80 kDa bilobal (N- and C-lobes) glycoprotein secreted from the liver into the blood, hTF binds Fe^{3+} very tightly (K_d ~10^{-22} M) [1], yet reversibly. Since hTF can bind iron in either or both lobes, four different hTF species (differing only in iron content) circulate in the blood: diferric (Fe_2hTF), monoferric N-lobe hTF (Fe_NhTF), monoferric C-lobe hTF (Fe_ChTF) and iron-free hTF (apohTF). At the pH of the serum (~7.4), Fe_2hTF binds with the highest affinity (K_d ~4 nM) to the specific transferrin receptor (TFR) located on the cell surface of all actively dividing cells [2]. The two monoferric hTFs (Fe_NhTF and Fe_ChTF) also form a stable high affinity complex with the TFR (K_d ~36 nM and K_d ~32 nM, respectively) [2]. In contrast, at neutral pH apohTF binds very weakly, if at all. After clathrin-mediated endocytosis of the hTF/TFR complex [3], the lower pH within the endosome (~5.6), in conjunction with salt and an, as of yet, unidentified chelator, stimulates the release of iron from hTF with the active participation of the TFR. Critically, apohTF binds with high affinity to the TFR at endosomal pH, allowing the apohTF/TFR complex to return to the cell surface instead of being targeted to late endosomes or to the lysosome for degradation [4]. Either through dissociation from the TFR or displacement by Fe_2hTF or the monoferric hTF species [5], apohTF is released into the serum at the cell surface, where it is free to bind more Fe^{3+} and repeat the cycle up to ~100 times [6].

The ubiquitously expressed TFR is a type II transmembrane homodimeric receptor which serves as the main route of entry for iron into most cells. Each of the ~90 kDa monomers of the TFR homodimer is comprised of a short cytoplasmic tail (residues 1–67) with an endosomal internalization motif, a single membrane-spanning portion (residues 68–88), a stalk region (residues 89–120) which contains two intermolecular disulfide bonds (Cys89 and Cys98) which covalently link the two monomers and a large extracellular ectodomain (residues 121–760) [7,8]. The hTF binding TFR ectodomain also referred to as the soluble portion of the TFR or sTFR (residues 121–760) is further subdivided into three domains: the protease-like domain (residues 121–188 and 384–606), the apical domain (residues 189–383) and the helical domain (residues 607–760) [9]. A stoichiometric 2:2 complex is formed between hTF and the TFR such that each TFR monomer binds one molecule of hTF [10]. Importantly, even in the absence of the stalk region, the sTFR in solution appears to always exist as a dimer due to the strong interaction of the helical domains of the two monomer with each other.

At any given time only 15–20% of the TFR molecules reside on the cell surface. An important and relevant question is whether the TFR is constitutively recycled or not, e.g., does the binding of hTF to the TFR promote internalization? The answer appears to be yes although this occurrence may be cell specific [11,12]. Thus it appears that HeLa cells undergo constitutive recycling whereas internalization of TFR on HL60 cells and both human and rabbit reticulocytes is influenced by hTF binding.

It is well established that because of their characteristically high rates of proliferation, cancer cells have dramatically increased iron requirements and therefore express an increased number of hTF receptors. This may be related to the fact that the rate limiting enzyme in DNA synthesis is iron containing ribonucleotide reductase [13]. A recent extensive review provides a detailed description of the many strategies used to target the hTF/TFR system [14]. As with all chemotherapy, the aim is to target the malignant cells/tumor while minimizing any detrimental effects on the organism as a whole. As an obvious example of this conundrum, reticulocytes within bone marrow actively synthesize iron containing heme used to carry oxygen. The increased number of TF receptors on reticulocytes makes them particularly vulnerable to the hTF/TFR targeting strategies and is likely to result in anemia. In this short review we focus on the structural and functional requirements that should be considered when attempting to use the hTF/TFR system for targeted therapeutics.

2. hTF/TFR specificity

Since the hTF/TFR interaction is critical to internalization and iron delivery within the cell, the molecular details of how the TFR interacts with its ligand, hTF, are clearly of great importance and must be carefully considered in developing hTF conjugates as targeted drug delivery vehicles. Until recently, a lack of precise structural details for the complex prevented an accurate consideration of the molecular interactions between hTF and the TFR. The first view of the TF/sTFR complex was a 7.5 Å resolution cryo-electron microscopy (EM) model published in 2004. Using the crystal structure of the unliganded sTFR and individually modeling in the available structures of the isolated human N-lobe and rabbit C-lobe (~85% similarity to the human C-lobe), this model suggested that the N-lobe is situated between the membrane and the TFR, and that the C-lobe makes significant contacts with the helical domain of the TFR (PDB ID: 1SUV) [15]. Due to the relatively low resolution and the ~9 Å shift with respect to the C-lobe required to fit the N-lobe into the existing density, this model provided a rather hazy picture of the interaction between hTF and the TFR. Availability of the structure of apohTF [16] and use of *in silico* modeling in conjunction with various mutagenesis studies of both hTF and the TFR resulted in a new model of hTF bound to the TFR [17]. When published, this computational model eliminated the physically impossible 9 Å gap between the N- and C-lobes of hTF, and was consistent also with the available mutagenesis data [2,18].

2.1. Structure of the hTF/sTFR complex

Publication of a relatively high resolution (3.22 Å) crystal structure of the Fe_NhTF/sTFR complex has now provided more precise molecular details of the interaction between the two binding partners [19]. Although this higher resolution structure is consistent with the relative orientation of the binding partners in the cryo-EM work, it provides considerably more detailed information about specific residues that are involved in the high affinity binding. The most important finding from this work is that the structure of the TFR undergoes significant changes as a result of the hTF binding. This was predicted by the Bjorkman group who had found that binding of the hemochromatosis protein, HFE to the TFR elicited structural changes in the TFR [20]. Because of the movements in the TFR when hTF binds, the cryo-EM model and the computational models based on the cryo-EM model have a number of inaccuracies with regard to the specific residues participating in the interaction. Nevertheless, similar to the cryo-EM model, three distinct binding motifs between hTF and the TFR are observed: the hTF N1-TFR motif, the hTF N2-TFR motif and the hTF C1-TFR motif. Obviously, covalent conjugation of a drug or toxin to residues involved in any of these three motifs could have detrimental effects on the hTF/TFR interaction and limit the actual amount of drug or toxin delivered to targeted cells. Furthermore, the interacting residues are surface exposed and therefore might be particularly susceptible to conjugation depending on the chemistry used.

2.1.1. hTF N1-TFR motif

The hTF N1-TFR motif is predominately comprised of backbone–backbone interactions between residues in hTF and the TFR [19]. In a competitive immunoassay, the R50A Fe_2hTF mutant was unable to effectively compete with biotinylated Fe_2hTF for binding to immobilized sTFR; binding was reduced by 70% [21]. This significant decrease in the apparent binding affinity of the R50A hTF mutant for the sTFR demonstrated that the N1-TFR interaction is strengthened by the presence

Download English Version:

https://daneshyari.com/en/article/2071079

Download Persian Version:

https://daneshyari.com/article/2071079

Daneshyari.com