



Improving the systemic drug delivery efficacy of nanoparticles using a transferrin variant for targeting

Ricky Y.T. Chiu^a, Takuma Tsuji^b, Stephanie J. Wang^a, Juntian Wang^a, Christina T. Liu^a, Daniel T. Kamei^{a,*}

^a Department of Bioengineering, University of California, Los Angeles 90095, USA

^b Department of Materials, Physics, and Engineering, Nagoya University, Nagoya, Japan

ARTICLE INFO

Article history:

Received 8 October 2013

Accepted 31 January 2014

Available online 10 February 2014

Keywords:

Transferrin

Mathematical modeling

Intracellular trafficking

Cellular association

Targeted drug delivery

ABSTRACT

Targeted therapy for the treatment of cancers using nanoparticles (NPs) decorated with transferrin (Tf) has been relatively successful, as several such nanocarriers are currently undergoing clinical trials. However, since native Tf has a low probability of delivering its payload due to its short residence time in the cell, or low cellular association, there is room to significantly improve the potency of current systems. We pioneered the redesign of this targeting ligand by altering the ligand–metal interaction, as suggested by our mathematical model, and here we present the first study to investigate the enhanced therapeutic efficacy of NPs conjugated to our engineered oxalate Tf. Our mathematical model was first used to predict that NPs conjugated to oxalate Tf will exhibit a higher degree of cellular association compared to native Tf-conjugated NPs. Our *in vitro* trafficking experiments validated the model prediction, and subsequent *in vitro* and *in vivo* efficacy studies demonstrated that this increase in cellular association further translates into an enhanced ability to deliver chemotherapeutics. Our findings signify the importance of the cellular trafficking properties of targeting ligands, as they may significantly influence therapeutic potency when such ligands are conjugated to NPs. Given the early success of a number of native Tf-conjugated NPs in clinical trials, there is potential for using Tf-variant based therapeutics in systemic drug delivery applications for cancer treatment.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Since current standard chemotherapy often leads to severe side effects, recent efforts have focused on cancer therapeutics demonstrating specificity towards the tumor and reduced toxicity to normal tissues. Tf has been used as a cancer-targeting agent in multiple delivery systems since the transferrin receptor (TfR) is overexpressed in many types of cancer cells. The investigation of CALAA-01, a Tf-conjugated cyclodextrin polymer-based nanoparticle, achieved the first targeted delivery of siRNA in a Phase I clinical trial in May 2008 [1,2]. Additionally, MBP-426, a Tf-bound liposome containing the cytotoxic platinum-based drug oxaliplatin, has proved promising, as it has completed Phase I clinical trials for solid tumors and is currently being evaluated in Phase II studies [3]. These nanocarriers, some of which are NPs, exploit the enhanced permeability and retention (EPR) effect to passively target tumors when administered systemically [4–6]. Once these NPs have accumulated inside the tumor, they can actively target cancer cells via the Tf decorated on their surfaces. Due to the overexpression of TfR, the Tf-conjugated NPs (Tf-NPs) can specifically internalize into cancer cells and deliver their drug payloads.

Although the conjugation of native Tf improves tumor-specific cellular uptake, significant limitations in Tf's efficacy as a drug carrier still exist since an iron-bound Tf molecule (holo-Tf) is restricted to a single 5 to 10 minute passage through a cell [7,8]. Once holo-Tf binds TfR, becomes internalized via receptor-mediated endocytosis, and releases iron, iron-free Tf (apo-Tf) is then recycled to the cell surface, where it quickly dissociates from TfR. Since rebinding of iron by Tf is an inefficient process, recycled Tf is often assumed to not be able to rebind iron and, therefore, TfR, thus limiting the amount of payload delivered by the NP.

In order to increase the probability of delivering therapeutics to TfR-overexpressing cancer cells, we wanted to increase the time Tf remained within a cell, i.e., increase its cellular association. Using a mathematical model that we developed for the Tf/TfR cellular trafficking pathway [8], we discovered that inhibiting the iron release from Tf in the endosome could increase the cellular association of Tf. To verify this prediction, we previously generated a variant of Tf by replacing the synergistic carbonate anion of Tf with oxalate, since oxalate can stabilize the iron atoms in Tf and therefore decrease Tf's iron release rate in the endosome [9]. Intracellular trafficking experiments with various cancer cell lines demonstrated that oxalate Tf exhibited an increased cellular association compared to native Tf, which led to enhanced delivery of a conjugated drug, diphtheria toxin [10]. Subsequently, site-directed mutagenesis was utilized to engineer Tf mutants that exhibited even slower iron release rates. Accordingly, conjugates of these Tf

* Corresponding author at: 5121 Engineering V, University of California, Los Angeles, CA 90095. Tel.: +1 310 206 4826; fax: +1 310 794 5956.

E-mail address: kamei@seas.ucla.edu (D.T. Kamei).

mutants with diphtheria toxin possessed even greater potency in *in vitro* cytotoxicity experiments with ovarian cancer [11] and glioblastoma cell lines. Moreover, intratumoral injections into xenografted glioma tumors in a mouse model resulted in near-complete tumor regression within 8 days [12].

Based on the greater cellular association and efficacy of our molecular oxalate and mutant Tf-drug conjugates, the work presented in this paper is a natural extension of our previous research with Tf variant systems and corresponds to the first investigation of the drug carrier properties of NPs conjugated to a Tf variant. In contrast to our laboratory's molecular drug conjugates, which are very promising for cancers treated locally, oxalate Tf-NPs administered intravenously may prove to be more suitable for most types of cancers, as they can avoid the competitive inhibition associated with endogenous Tf present at higher concentrations in the blood serum (3–6 μM) [8]. More importantly, our novel oxalate Tf-NPs and Tf variant-drug conjugates are similar in that both may bypass native Tf's rapid recycling (Fig. 1). Specifically, we

hypothesized that oxalate Tf-NPs would be able to significantly improve upon the status quo.

To test our hypothesis, we performed a number of theoretical and experimental studies. An extension of our previous mathematical model predicted an increased cellular association for oxalate Tf-NPs, and this prediction was experimentally verified via cellular trafficking studies with radiolabeled Tf-conjugated polystyrene NPs (Tf-PNPs). To see if this improvement could be translated into increased potency with drug-loaded NPs, doxorubicin (DOX)-loaded poly(lactide-co-glycolide) (PLGA) nanoparticles (DP) were prepared and then conjugated with polyethylene glycol (PEG) and Tf to form Tf-PEG-DPs (TPDPs). The variant TPDP demonstrated increased potency over the native Tf-system in *in vitro* cytotoxicity assays. Finally, through *in vivo* experiments with a mouse model, we showed that using a single, intravenous injection of variant TPDP led to a much greater inhibition of tumor growth compared with native TPDP and the phosphate-buffered saline (PBS) control.

2. Materials and methods

2.1. Mathematical model of Tf-NP/TfR trafficking pathway

We extended the Tf/TfR trafficking model previously developed by our laboratory [10]. This extended model, which was derived using the principles of mass action kinetics, describes the trafficking behavior of Tf-NP, including its binding to cell-surface receptors, internalization, partitioning in the endosomal compartment, recycling, and degradation. To transition from describing the trafficking behavior of molecular ligands to that of nanoparticles, two main parameters from the previous model were altered, namely, the equilibrium dissociation constant (K_D) of Tf-NP binding to TfR and the partitioning of the drug carrier in the endosomal compartment.

The system of ordinary differential equations was solved using the modeling software Berkeley Madonna. The length of the model simulations was set to 2 h with initial conditions set to zero for all species except the concentration of holo Tf-NP in the media (0.75 pM) and the number of TfR on the cell surface. The area under the curve (AUC) of internalized Tf-NPs versus time was calculated and used as the metric to quantify the cellular association of Tf-NPs. This was the same metric as the one used in our previous mathematical model [10].

2.2. Cell culture

PC3 human prostate cancer cells were a kind gift from Dr. Lily Wu (University of California at Los Angeles Pharmacology, Los Angeles, CA). A549 human non-small cell lung cancer cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Both cells were seeded onto 75 cm² tissue culture flasks (Corning Incorporated, Corning, NY) and grown in RPMI 1640 (for PC3 cells, Invitrogen, Carlsbad, CA) or F12K (for A549 cells, Invitrogen) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin/streptomycin (Invitrogen) at a pH of 7.4. The cells were incubated in a humidified atmosphere with 5% CO₂ at 37 °C. All cell lines used in this study were authenticated by Laragen, Inc. (Culver City, CA) using the Promega StemElite (Promega) for STR analysis. Results indicated that the cell lines used in this study are authentic. All reagents and materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

2.3. Conjugation of Tf to PNP

PNP (100 nm diameter) were purchased from Phosphorex, Inc. (Fall River, MA) with free surface amines and encapsulated with a proprietary hydrophobic dye. Apo-transferrin (apo-Tf) was purchased from Sigma-Aldrich (St. Louis, MO). The molecules 2-iminothiolane

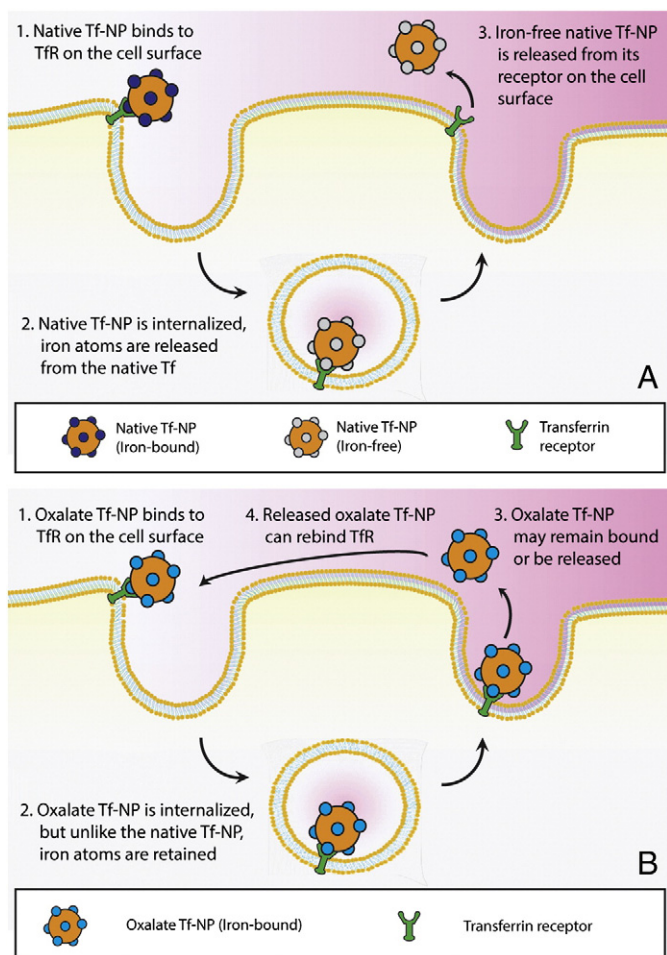


Fig. 1. Schematic of the native and oxalate Tf-NP/TfR trafficking pathways. (A) Iron-bound native Tf-NP binds TfR, is internalized, and releases iron once it enters the acidic environment (pH ~ 5.6) of the endosome. Iron-free Tf-NP is then recycled to the cell surface, where it quickly dissociates and is assumed to be lost, due to the inefficiency with which Tf-NP rebinds iron. (B) Oxalate Tf-NP is internalized, but exhibits inhibited iron release in the acidified endosomal compartment. Iron-bound Tf-NP, instead of iron-free Tf-NP, returns to the cell surface. Since iron-bound Tf-NP retains its high affinity for TfR at the cell surface, it exhibits a slow dissociation rate. As a result, iron-bound Tf-NP can either remain bound to TfR and re-enter the cellular trafficking pathway or, if dissociation occurs, quickly rebind another TfR for internalization. Accordingly, cellular association is increased by increasing the number of cycles, not by lengthening the residence time of one cycle. For simplicity, only monovalent Tf-TfR binding is depicted in this figure, but multiple Tf on a Tf-NP can exhibit multivalency effects and bind to multiple TfR on the cell surface.

Download English Version:

<https://daneshyari.com/en/article/7864760>

Download Persian Version:

<https://daneshyari.com/article/7864760>

[Daneshyari.com](https://daneshyari.com)