Review

Drug delivery via the transferrin receptor-mediated endocytosis pathway

Qing Xia¹, Xiu-Wei Yang², Xiao-Da Yang¹,²*, Zhong-Ming Qian³, Kui Wang¹,²

1. Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China
2. The State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China
3. Laboratory of Iron Metabolism, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong

Abstract: The membrane transferrin receptor-mediated endocytosis has been exploited for developing novel targeted drug delivery systems, which could have a variety of applications in the site-specific delivery of anticancer drugs, proteins and therapeutic genes into proliferating malignant cells that overexpress the transferrin receptors. This is achieved by coupling transferrin or monoclonal antibody to transferrin receptor with therapeutic drugs or drug delivery vesicles. The transferrin conjugates can be obtained by use of either bifunctional chemical linkers or by genetic infusion of therapeutic peptides/proteins into the structure of transferrin / and monoclonal antibody to transferrin receptor. A variety of drug carriers such as liposomes, nanoparticles and DNA-polymers complexes (i.e. polyplex and lipoplex) were used to efficiently deliver the transferrin conjugates. Use of transferrin conjugates results in improvement in drug efficacy, selectivity and drug release as well as reduction in drug toxicity. This paper reviews the basic biochemistry of transferrin and the transferrin receptor as well as the strategy for developing targeted drug delivery system.

Keywords: Transferrin; Transferrin receptor; Drug delivery


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1. Introduction

The rapid progress of current pharmaceutical drug discovery has resulted in the emergence of increasing numbers of novel therapeutic drugs, especially biological drugs, for the treatment of a variety of diseases. However, one major problem associated with systemic drug administration is the lack of specific affinity of a drug toward a pathological site. Polymer-based and liposome-based delivery systems have the potential as specific and target-oriented delivery systems¹,² to enhance the therapeutic index of drugs. However, delivery systems utilizing the mechanism of receptor-mediated endocytosis (RME) are becoming an attractive option to improve the permeability, retention and target specificity of macromolecular drugs. The level of transferrin (Tf) receptor expression on the tumor cells or other abnormal cells is higher than that on the normal cells. The transferrin/Tf receptor pathway has been shown to be of great potential in the delivery of anticancer drugs, proteins and therapeutic genes into proliferating primary malignant cells³–⁵.

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*Corresponding author. Tel.: 86-10-862583631; e-mail: xyang@bjmu.edu.cn
2. Transferrin receptor-mediated endocytosis

2.1. Transferrins

Transferrins are single-chain glycoproteins containing 700 amino acids (80 kDa). The sequence identity between different species and different members of the family is extremely high. For example, there is 78% identity between rabbit and human serum transferrin, 60% between serum transferrin and lacoferrin, and 40% identity between melanotransferrin and other transferrins. A variety of crystal structures of transferrins are available and exhibit great similarity[6]. The polypeptide chain is folded into two structurally similar but functionally different lobes, referred to N- and C-lobe, respectively. Each lobe has two domains enclosing a deep hydrophilic cleft bearing an iron-binding site. Bicarbonate is essential for strong binding of iron to the specific site of Tf and may also have a role in iron release. In addition to iron, many metal ions other than iron have been found to bind to the specific iron sites[7], thus Tf has been implicated in the transportation of other metal ions.

Figure 1 shows the crystal structure of the transferrin and transferrin receptor complex.

2.2. Transferrin receptors

There are two major types of transferrin receptors. The transferrin receptor 1 (TfR1) appears to be expressed in all nucleated cells in the body[8]. TfR1 is over expressed on rapidly dividing cells[9]. In contrast, expression of TfR1 is low or frequently undetectable in non-proliferating cells, The TfR1 is a transmembrane homo-dimer that can bind up to two molecules of Tf. The binding affinity of Tf for various receptors is very high (10^5 to 10^10 M^-1)[6]. Crystallographic studies of the ectodomain of human TfR1 (residues 122 to 760) revealed that the homo-dimer of TfR1 is organized as a butterfly-like shape, as shown in Figure 2.

The transferrin receptor 2 (TfR2) share a 45% identity and 66% similarity in its extracellular domain with TfR1. The expression of TfR2 is not regulated by IRP-mediated feedback regulatory mechanism in response to cellular iron status. Instead, it may be regulated by a mechanism, probably related to the cell cycle or cellular proliferation status[10]. The high level of TfR2 expression in the liver suggests TfR2 possibly contributes to the liver’s ability to regulate iron homeostasis[11,12]. The affinity of TfR2 for iron-loaded Tf is 25-fold lower than that of TfR1 for Tf[13].

2.3. Transferrin receptor-mediated endocytosis and iron uptake

Transferrin receptor-mediated uptake of transferrin-bound iron is one of the best-understood processes in cell biology[13,14]. Briefly, the process is triggered by the binding of Fe_{2+}-Tf to a specific cell-surface TfR1. After endocytosis via clathrin-coated pits, which bud from the plasma membrane as membrane-bound vesicles or endosomes, the Fe_{2+}-Tf-TfR1 complex is routed into the endosomal compartment. Upon maturation and loss of the clathrin coat, the endosome becomes competent to pump protons in a process energized by ATPase, and the endosomal lumen is rapidly acidified to a pH of about 5.5. At this pH, the binding of iron to Tf is weakened, leading to iron release from the protein. The free Fe^{3+} released to endosomes is reduced to Fe^{2+} on the cis-side of the endosomal membrane probably mediated by oxidoreductase. Fe^{2+} is subsequently transported out of the endosome by the divalent metal transporter DMT1. After release of iron, the resultant apo-Tf: TfR1 complex is then recruited through exocytic vesicles back to the cell surface. At extracellular physiological pH of 7.4, apo-Tf dissociates from its receptor due to its low affinity at this pH and is released into the circulation, and reutilized.
3. Drug delivery based-on transferrin receptor-mediated endocytosis

Since TfR is upregulated in rapidly dividing cells, it provides the opportunity to use it for tumor-targeted delivery. Therefore, transferrin and anti-TfR antibody have long been explored for development of delivery systems for anticancer agents and genes, and also to transfer drugs across a variety of biological barriers via transferrin-mediated transcytosis pathways. Transferrin based drug delivery system can achieve site specific delivery of anticancer agents and circumvent multi-drug resistance, etc.

3.1. Transferrin as a metallodrug mediator

In human serum, the transferrins are primarily iron-binding proteins. Because Tf is only about 30% saturated with iron, there is still potential capacity for binding to other metal ions present in the body. Indeed, over 30 metal ions have been reported to bind to Tf. Such binding may play an important role in the transport and delivery of medical diagnostic radioisotopes such as $^{67}$Ga$^{3+}$ and $^{111}$In$^{3+}$ and therapeutic metal ions such as Bi$^{3+}$, Ru$^{3+}$ and Ti$^{4+}$.

3.2. Transferrin conjugates in site-specific drug delivery

There are many ways to prepare transferrin conjugates. A simple method is the direct coupling of therapeutic agents to Tf by a covalent bond. When the therapeutic agents are biological molecules, incorporation of the drug into the structure of Tf using recombinant protein engineering may be of great merit. Moreover, the biotin-avidin system can be a universal strategy to establish the transferrin-drug conjugates for site targeting. The avidin/biotin bond is not covalent in nature, but binding is extremely strong with a dissociation constant ($K_d$) in the order of $10^{-15}$ M and dissociation half life of about 89 d. Although the avidin/biotin bond is stable in the circulation, it is labile at tissue depot sites. Therefore, the avidin/biotin system is ideal for drug delivery to tissues.

To overcome the problem that the native transferrin in circulation may compete with transferrin-drug conjugates, coupling of therapeutic agents to anti-TfR antibody (or its Fab fragment) has been frequently employed for preparation of the conjugates.

3.2.1. Transferrin-drug conjugates

Many transferrin conjugates of various therapeutic agents have been prepared for specific drug delivery (Table 1). Artemisinin tagged to transferrin via carbohydrate chain has been shown to have high potency and specificity against cancer cells. Doxorubicin (Adriamycin) is an effective and widely used cancer chemotherapeutic agent; however, cardio-toxicity and emergence of resistance significantly limit its utility in clinical practice. Significantly, Tf-doxorubicin conjugate was 4–5 times more potent than free drug in doxorubicin sensitive tumor cell

<table>
<thead>
<tr>
<th>Table 1. Conjugates of therapeutic agents with transferrin</th>
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<tr>
<td>Therapeutics</td>
</tr>
<tr>
<td>Doxorubicin</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
</tr>
<tr>
<td>Liposome-PEG</td>
</tr>
<tr>
<td>Polymeric</td>
</tr>
<tr>
<td>Chitosan vesicles</td>
</tr>
<tr>
<td>Tf-PEG-Liposome</td>
</tr>
<tr>
<td>Toxin</td>
</tr>
<tr>
<td>CRM107</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>Oxpalatin</td>
</tr>
<tr>
<td>Chlorambucil</td>
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<tr>
<td>γ-IFN</td>
</tr>
<tr>
<td>Paclitaxel</td>
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<tr>
<td>Yeast FCU1</td>
</tr>
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F(ab’)$_2$, anti-tetanus fragments; MMC, mitomycin C.
lines such as HL60, Hep2 in vitro and 5 and 10 times more potent in resistant cell lines[39]. For highly multidrug-resistant cells, the conjugate inhibited the cell proliferation with IC₅₀ of 0.025–0.2 mM, while doxorubicin did not exert any cytotoxicity even at 1 mM concentration[40].

Chlorambucil (leukeran), another anticancer drug used clinically against chronic lymphatic leukemia, lymphomas and advanced ovarian and breast carcinomas, is limited by its toxic side effects. The Tf-chlorambucil conjugate exhibited 3 to 18-fold enhancement of activity in the MCF7 mammary carcinoma and MOLT4 leukemia cell line, but this conjugate can be administrated at higher doses compared with unbound chlorambucil[34].

Tf-CRM107 is a conjugate protein of diphtheria toxin with a point mutation (CRM107) linked by a thioester bond to human transferrin. This conjugate exhibits potent in vitro cytotoxicity against mamalian cells expressing the transferrin receptor with activity at picomolar concentrations[41].

3.2.2. Transferrin and transferrin antibody coupled liposomes/nanoparticles

Liposome and nanoparticle are potent and popular carrier systems for controlled drug release. Modification of their surface with transferrin or anti-TfR antibody was shown to improve the stability, prolong the period of drug release, and most importantly increase the target specificity and drug permeability. Therefore, vast amounts of work have focused on the development of this actively targetable particle/liposome. A small unilamellar mercaptoundecahydrododecaborate (BSH)-encapsulated, transferrin-conjugated polyethylene glycol liposomes (Tf-PEG liposomes) was prepared[42]. Intravenous injection of Tf-PEG liposomes was shown to increase the tumor retention of the drugs. Studies on the tissue selectivity of liposomes coated with transferrin-coupled polyethylene glycol revealed that Tf-dependent uptake in liver and brain was dependent on the size of the liposomes used[43]. Actively targetable nanoparticles (ATN) and PEG-coated biodegradable polycyanoacrylate nanoparticles (PEG-nanoparticles) conjugated to transferrin were developed for paclitaxel delivery[38]. The efficacy of paclitaxel-loaded biodegradable nanoparticles on tumor inhibition was determined in human prostate cancer cell line (PC3) and in a murine model of prostate cancer[37]. The IC₅₀ of the drug with Tf-conjugated nanoparticles (Tx-NPs-Tf) was about 5-fold lower than that with unconjugated form or the drug in solution. Animals that received a single-dose intratumoral injection of Tx-NPs-Tf (Tx dose = 4 mg/kg) demonstrated complete tumor regression and a greater survival rate than those that received either Tx-NPs or Tx-Cremophor EL formulation. Linear and cyclodextrin-based polymers were complexed with DNAzyme molecules to form sub-50 nm particles termed ‘polyplexes’[44]. These transferrin-modified nanoparticles were employed for targeted delivery of RNA-cleaving DNAzyme (short catalytic single-stranded DNA molecules) to tumor tissue. DNAzymes packaged in polyplex formulations were taken up by tumor cells upon intravenous bolus injection. The polyplex formulations could be concentrated and retained in tumor tissue and other organs, whereas unformulated DNAzyme was eliminated from the body within 24 h post-injection.

After being taken up by the cells, the transferrin-drug conjugates should follow intracellular pathways of receptor-mediated endocytosis. To optimize drug delivery, control of intracellular trafficking was studied. A pH-sensitive fusogenic peptide, GALA (WEAALAEALAEALAEALAEALAEALAA) was introduced to transferrin-modified liposomes[45]. When GALA was introduced into liposomal membranes, the encapsulated rhodamines were efficiently released and diffused into the cytosol. Cross-linking of TfR induced by oligomeric Tf binding was also shown to alter the intracellular trafficking of Tf-TfR complexes[46]. Methotrexate was conjugated to Tf-oligomer and it maintain specificity of the TfR-binding, however, cross-linking of TfR redirects Tf-oligomers out of the recycling pathway, and targets them to intracellular degradation in cultured tumor cells.

3.3. Transferrin in gene delivery

The TfR-mediated gene delivery has an attractive feature since it provides an opportunity to achieve cell specific delivery of DNA complex that might also enhance the transfection efficiency. HVJ (hemagglutinating virus of Japan; Sendai virus) containing the F-transferrin chimeric protein vector demonstrated 32-fold greater tumor-targeting efficiency than wild-type HVJ envelope (HVJ-E) vector[47]. Since DNA is a large polyanionic molecule, many different polycationic molecules have been used for
this purpose, including polyornithine, histones, poly-
ethyleneimine. Polycationic molecules and DNA can
form stable molecular complexes (‘polyplexes’), which are then conjugated with a cell-binding ligand
transferrin or OX26 mAb to achieve receptor-
mediated endocytosis.

Transferrin-polyethylene glycol-polyethyleneimine/ DNA complex is prepared for systemic tumor-targeted
gene transfer\[48\]. However, polylysine is the most
widely used polycation since it is available in a large
variety of molecular weights and can also be easily
degraded by cells\[49\]. Tf-PLL-DNA conjugates pro-
vide a very efficient vector for gene transfer in some
tissue culture cells. In K-562 cell line, virtually
100% of the cell population was found to express
the transfected reporter gene for a protracted period
of days\[50\].

Cationic liposomes are composed of positively
charged lipid bilayers that can be complexed to
negatively charged, naked DNA by simple mixing.
The resulting cationic liposomes-DNA complexes
(lipoplexes) formed by a combination of electro-
static attraction and hydrophobic interaction have
been used extensively as non-viral vectors for the
intracellular delivery of reporter or therapeutic genes
in culture and in vivo\[51\]. Association of Tf with lipo-
plexes significantly overcame the inhibitory effect of
serum and facilitated efficient transfection in many
cell lines, including HeLa, K562 cells and lung car-
cinoma cells Calu3, H292 cells\[52,53\]. The structure
and formation process of this efficient and effica-
cious Tf-lipoplex has been elucidated\[54\].

In addition to polyplexes-transferrin conjugate and
lipoplexes-transferrin conjugate, other vectors have
included coupling of genes to bioninylated Tf via
streptavidin bridge\[55\]. There is no size limitation for
the gene to be conjugated and the conjugates con-
structed in such a way are stoichiometrically con-
trollable and were shown to have the potential in in
vivo gene delivery. Gene delivery systems based on
the use of Tf modified nanoparticales have also been
developed\[56\].

However, Tf transcytosis gene delivery still suffers
from a lower efficiency compared with viral vectors.
Future work needs to focus on enhancing transfec-
tion efficiency that may include synthesis of novel
bifunctional carriers such as inorganic nanorods,
chemically modifying the system, such as optimizing
parameters affecting surface binding and association
and developing a specific mechanism to effectively
release therapeutic genes from the endosome into
the cytosol.

3.4. Other applications

The transferrin conjugate drug delivery systems are
also applied to improve drug delivery across blood
brain barrier (BBB) and improve the absorption of
drugs by oral administration or via the pulmonary
route. Insulin-transferrin conjugate was developed
for treatment of diabetes. It was shown that oral ad-
ministration of insulin-transferrin conjugate permeates
across the caco-2 cells through transferrin receptor-
mediated transcytosis and exhibit hypoglycemic
effect in diabetic rats\[57\]. The possibility of using
TfR-mediated transcytosis for systemic delivery of
therapeutic proteins via the alveolar epithelium was
examined using cultured primary rat alveolar epithelial
cell monolayers\[58\]. A nanogel encapsulated oligonu-
cleotides with surface modified with transferrin can
be effectively transported across the BBB through
the transcellular pathway in an in vitro model\[59\].

4. Summary

In the past few decades, intensive studies have been
made toward understanding Tf trafficking and Tf/TfR
mediated cellular iron uptake pathway. The strategy
of exploiting Tf/TfR as a drug carrier/delivery system
is based on elevated levels of transferrin receptors
present on the surface of tumor cells. The Tf/TfR
delivery system also increases the permeability
across a variety of biological barriers, e.g. BBB and
intestinal mucosa. Some of the expected advantages
of Tf-drug conjugates are a preferable tissue distri-
bution, prolonged half-life of the drug in the plasma,
and controlled drug-release from the conjugates. A
vast number of Tf-conjugated systems for delivering
therapeutic drugs/genes including small molecule
anticancer drugs, peptides or proteins have been
developed. Results indicate that the well-tunable
stoichiometric properties and stability of the conju-
gates by using different linkage strategies make the
targeted drug delivery feasible via the Tf-mediated
endocytosis pathway.

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转铁蛋白-转铁蛋白受体系统在药物运输中的应用

夏青1, 杨秀伟2, 杨晓达2*, 钱忠明3, 王突1,2

1. 北京大学 药学院 化学生物学系 北京 100191
2. 北京大学 天然药物及仿生药物国家重点实验室 北京 100191
3. 香港理工大学 应用生物和化学技术系 铁代谢实验室 香港

摘要: 转铁蛋白是转铁蛋白受体介导的内吞作用应用于新的药物靶向给药系统, 包括将抗肿瘤药物、蛋白和治疗基因定位运输至恶性增殖细胞或是中枢神经系统。通过偶联转铁蛋白或其抗体连接到转铁蛋白受体上成为治疗的药物或转运载体。多种药物载体如脂质体、纳米颗粒与DNA复合物用于高效运输治疗物。转铁蛋白复合物的运用不仅提高了药效, 改善了选择性与药物释放性能, 降低了毒性, 同时也延长药物血浆半衰期, 提高药物主动靶性。本篇综述介绍了转铁蛋白/转铁蛋白受体介导的药物运输的研究进展。

关键词: 转铁蛋白; 转铁蛋白受体; 药物运输