Targeting with Transferrin

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1. INTRODUCTION

Transferrin, (trf) a serum glycoprotein (molecular weight 80,000) has been of interest to several investigators due to the high efficacy of its endocytosis via transferrin receptors. Its basic role in the body is to facilitate the transport of iron into cells. Iron is a universal requirement for cellular proliferation particularly for rapidly growing tumor cells. Since tumor cells are rapidly dividing, they have increased requirement of iron as compared to normal cells. Trf receptors are present in large numbers in both transformed and cancer cells (1-3). It is estimated that on an average more than 150,000–1000,000 trf receptors are present on the cell surface of tumor cells (4,5). Cancer cells undergo metastasis to distant sites and need iron to proliferate. The predominant source of iron in the human body is diferric trf. Trf is taken up by tumor cells by receptor-mediated endocytosis. Diferric trf is processed in endosomes where it releases its iron. The trf thus released at the cell surface is called apotransferrin, which can again bind two molecules of iron from the blood circulation to form diferric trf to start the process again (6,7).

Several anticancer drugs and toxins have been linked to trf via various chemical linkages. Some of these agents are Adriamycin (adr), methotrexate, ricin A, diphtheria toxin, pseudomonas exotoxin, whole ricin, etc. The rationale for linking the anticancer agents to trf was mostly for tumor targeting in an effort to minimize the adverse toxic effects observed with anticancer drugs and toxins. Other approaches that have been used currently are the use of liposomes (8,9), microspheres, polymers (10,11), and monoclonal antibodies (MAbs) (12-15).

This chapter will review the application of trf for targeting anticancer agents and toxins to tumor cells. Most of the review will cover the various methods of conjugation used to link trf to anticancer drugs and also to liposomes containing anticancer agents.

From: Cancer Drug Discovery and Development: Tumor Targeting in Cancer Therapy
Edited by: M. Pagé © Humana Press Inc., Totowa, NJ

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Some studies conducted with tumor-bearing BALB/c nude mice and Trf-adr conjugate will also be discussed. The latter part of this review will discuss about trf-ricin conjugates and gene delivery via trf.

**2. RECEPTOR-MEDIATED ENDOCYTOSIS VIA THE TRANSFERRIN RECEPTOR**

The trf receptor, which is a dimeric transmembrane glycoprotein of 180kDa, is present on the surface of most rapidly proliferating higher eukaryotic cells. The tissues expressing trf receptors are bone marrow, intestinal epithelium, and epidermis. However, some non-proliferating tissues also express trf receptors, e.g., hepatocytes, tissue macrophages, pituitary cells, pancreas islet cells, or the endothelium of brain capillaries (16,17).

The receptor-mediated endocytosis with trf involves its internalization, release of the iron at the low endosomal pH while apotransferrin remains bound to the receptor. The apotransferrin-receptor complex is then processed into the exocytic vesicles back to the cell surface where apotransferrin is released into the medium. This entire trf cycle takes only 4–5 min with a mean transit time of about 10 min. This rapid recycling leads to very high turnover numbers in the order of $2 \times 10^4$ trf molecules internalized per min/cell (18). Another pathway which has been proposed is routing via the trans Golgi apparatus and then back to the plasma membrane. Several studies have also been reported in which lysomotropic agents have been shown to strongly influence the intracellular fate of trf.

**3. TRANSFERRIN-LINKED DRUGS**

**3.1. Conjugation of Transferrin to adr via Schiff Base**

The method of conjugating trf to adr via a Schiff base was first reported by Yeh and Faulk (19). The method followed in our laboratory was the same with some minor modifications. Briefly 24 mg of human apotransferrin (Sigma Chemicals, MO) was dissolved in 1 mL of saline and added to a solution of adr (3 mg/mL). The mixture was stirred for 4 min and glutaraldehyde (21.5 mM) was added while stirring at room temperature. The reaction was stopped by adding 37.2 mM ethanolamine (470 µL in 150 mM NaCl and 10 mM HEPES, pH 8.0) and was further dialyzed against HEPES-buffered saline in the dark for over 24 h. The mixture was centrifuged at 4000rpm to remove any insoluble precipitate and the supernatant collected was passed through a Sepharose CL-6B column (Pharmacia, Uppsala, Sweden) and 1 mL fractions were collected with the help of a LKB-Fraction 100 collector. Elution was continuously monitored at 280 nm and 495 nm with a Pharmacia LKB VM 2141 UV monitor. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the conjugate-column fractions was performed with a vertical slab gel composed of a 7.5% (w/v) acrylamide.

The trf immunoreactivity with the glutaraldehyde conjugate was demonstrated by the method of indirect immunofluorescence. The crystal violet dye-uptake assay was used to assess the in vitro toxicity of the Trf-adriamycin (Trf-adr) conjugate.

**3.2. Conjugation of Transferrin to adr via cis-aconityl Spacer**

This method was used to link adr to Trf via a lysomotropic spacer cis-aconitic anhydride so that it can be cleaved in the acidic compartment of the lysosomal enzymes