Physiologically Based Pharmacokinetic Model for T84.66: A Monoclonal Anti-CEA Antibody

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ABSTRACT: Antibodies directed against tumor associated antigens are being increasingly used for detection and treatment of cancers; however, there is an incomplete understanding of the physiological determinants of antibody pharmacokinetics and tumor distribution. The purpose of this study is to (a) compare the plasma pharmacokinetics of T84.66, a monoclonal anti-CEA antibody directed against tumor associated carcinoembryonic antigen (CEA), in control and CEA expressing LS174T xenograft bearing mice, and (b) to develop a physiologically based pharmacokinetic (PBPK) model capable of integrating the influence of CEA and the IgG salvage receptor, FcRn, on T84.66 disposition. T84.66 pharmacokinetics were studied following i.v. administration (1, 10, 25 mg/kg) in control and xenograft bearing mice. In control mice, no significant differences in clearance were observed across the dose range studied. In mice bearing xenograft tumors, clearance was increased by four- to sevenfold, suggesting the presence of a "target mediated" elimination pathway. T84.66 plasma disposition was characterized with a PBPK model, and the model was applied to successfully predict antibody concentrations in tumor tissue. The PBPK model will be used to assist in the development of antibody-based targeting strategies for CEA-positive tumors. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:1582–1600, 2010

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INTRODUCTION

Monoclonal antibodies[1] have been investigated for use as "magic bullets" to target a desired site of action, while minimizing unwanted side effects. The number of antibodies approved in the oncology area has steadily increased over recent years and, at present, nine monoclonal antibodies have been FDA-approved for use in cancer treatment. More than 20 antibodies aimed at different antigen targets are presently in clinical oncology trials.[2]

Immune gamma globulin (IgG) is the most prevalent antibody isotype found in man, with average serum concentrations in the range of 10–12 mg/mL.[3] Virtually all mAb that are in development are IgG antibodies. Compared to other immunoglobulin isotypes, IgG has the longest half-life and lowest fractional catabolic rate.[4] In 1964, Brambell proposed that specific transport proteins were capable of binding to IgG and limiting its elimination. With increasing IgG concentrations, these receptors would get

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saturated, and the excess unbound IgG would face catabolism.[5] Further research has led to the isolation and cloning of this transport protein, and it has been named "FcRn" or the Fc-receptor of the neonate.[6,7] Various groups have now demonstrated the role of FcRn as a salvage receptor protecting IgG from elimination.[8–10] FcRn has been shown to be expressed in the vascular endothelial cells of various tissues and plays a key role in maintaining IgG homeostasis. However, in addition to FcRn, interaction of an antibody with its target antigen may be a very important determinant of antibody disposition.[11]

A number of FDA-approved mAb demonstrate "target mediated elimination,"[12] where antibody elimination is mediated by specific binding to targets such as epidermal growth factor receptor (EGFR), CD33, CD11 and Her2.[11,13] Carcinoembryonic antigen (CEA)[14] is one of the most widely studied tumor-associated antigens, and it is known to be expressed at low levels in normal tissues such as epithelial cells of the esophagus, pancreas, uterus and prostrate, mucous stomach cells and ducts of sweat glands.[15–18] CEA levels are increased by several fold in a wide range of adenocarcinomas including breast cancers, colorectal cancers, and other cancers of the gastrointestinal tract. Their differential expression offers a possibility for discrimination between normal tissues and tumor cells, making CEA an extremely attractive target for antibody-directed tumor imaging[19–21] or drug targeting.[22,23] However, the influence of tumor-associated CEA on the plasma pharmacokinetics of anti-CEA antibodies has not been thoroughly investigated.

In this work, we have developed a physiologically based pharmacokinetic (PBPK) model incorporating a target-mediated disposition component to examine the influence of antigen–antibody interaction on antibody pharmacokinetics. The model also incorporates additional, known complexities associated with antibody disposition such as: convective uptake into tissues, FcRn-mediated protection within endosomes of the vascular endothelium, and the influence of endogenous antibody levels on the FcRn-mediated transport of therapeutic antibody. Plasma concentration data were collected from mice bearing tumors expressing CEA and control mice lacking the tumor-associated antigen. The PBPK model was able to capture the plasma data in control mice and in antigen-positive tumor-bearing mice, and the model was shown to predict T84.66 antibody concentrations within tumor tissue.

MATERIALS AND METHODS

Production and Purification of T84.66

Hybridoma cells producing T84.66, a monoclonal anti-CEA antibody, were purchased from the American Type Culture Collection (ATCC # HB-8747, Manassas, VA). T84.66 is known to bind CEA with an equilibrium dissociation constant of 3.8×10^{-11} M.[24] For the purposes of antibody production, cells were grown in 1L spinner flasks containing serum free media (Hybridoma SFM, Invitrogen, NY), and culture supernatant was harvested 2–3 times weekly. Anti-CEA antibody was purified from culture supernatant by protein G chromatography (Amersham Biosciences, Uppsala, Sweden) by use of a Bio-Rad medium pressure chromatography system (Bio-Rad, Hercules, CA).

LS174T Adenocarcinoma Cells

LS174T human colon cancer cells (ATCC# CL-188, Manassas, VA), which are known to express CEA, were cultured in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (Invitrogen, Grand Island, NY). Cells were detached by tapping culture flasks, suspended in sterile saline, counted, and used to establish xenografts.

Animals

Male athymic nude mice (20–25 g, 5–6 weeks old) were obtained from Harlan (Indianapolis, IN). Mice were housed in a sterile room, handled under aseptic conditions in a laminar hood, and fed autoclaved chow. 100 μL of LS174T cells in suspension ($\sim 5 \times 10^6$ cells) were injected s.c. into the right flank of the mice. Mice were monitored regularly to check for tumor growth and body weight. The tumor size was measured by vernier calipers, and tumor volume was defined by the standard formula; $l \times w^2/2$, where l represents the length of the longest diameter (mm) and where w represents the length of the axis perpendicular to l. All animal procedures were approved by the Institutional Animal Use and Care Committee of the University at Buffalo.

Pharmacokinetic Study

T84.66 was administered intravenously via penile vein injection to xenograft-bearing mice (starting