Studies of Nontarget-Mediated Distribution of Human Full-Length IgG1 Antibody and Its FAb Fragment in Cardiovascular and Metabolic-Related Tissues

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ABSTRACT: Tissue distribution and pharmacokinetics (PK) of full-length nontargeted antibody and its antigen-binding fragment (FAb) were evaluated for a range of tissues primarily of interest for cardiovascular and metabolic diseases. Mice were intravenously injected with a dose of 10 mg/kg of either human IgG1or its FAb fragment; perfused tissues were collected at a range of time points over 3 weeks for the human IgG1 antibody and 1 week for the human FAb antibody. Tissues were homogenized and antibody concentrations were measured by specific immunoassays on the Gyros system. Exposure in terms of maximum concentration (C_{max}) and area under the curve was assessed for all nine tissues. Tissue exposure of full-length antibody relative to plasma exposure was found to be between 1% and 10%, except for brain (0.2%). Relative concentrations of FAb antibody were the same, except for kidney tissue, where the antibody concentration was found to be ten times higher than in plasma. However, the absolute tissue uptake of full-length IgG was significantly higher than the absolute tissue uptake of the FAb antibody. This study provides a reference PK state for full-length whole and FAb antibodies in tissues related to cardiovascular and metabolic diseases that do not include antigen or antibody binding. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1825–1831, 2015

Keywords: pharmacokinetics; distribution; diffusion; IgG antibody; nontargeted full-length antibody; nontargeted FAb antibody; immunoassay; perfusion; metabolic diseases; clearance; mathematical model

INTRODUCTION

For many antibodies and other targeted drug modalities, the molecular target is located within tissues and their pharmacodynamics (PD) and exaggerated PD (toxicity) will be a function of tissue concentration. Therefore, it is important to understand the factors associated with distribution of antibody-based therapeutics for optimal design of *in vivo* studies to adjust the pharmacological effect(s).

The process of distribution can be characterized by volume of distribution, influenced by physiological variables such as binding in blood or plasma and tissue, partition into fat, body composition, and body size.[1,2] Specifically, volume of distribution of an antibody relates to the volume of plasma or blood, the volume of tissue, and the tissue-to-plasma partitioning. As a base case, whole IgG antibodies are primarily distributed into the plasma compartment. However, as binding to plasma or tissue targets can influence antibody distribution, the density and expression of the target antigen as well as the active transport processes of IgG by the neonatal Fc receptor (FcRn) across the neonatal intestine may also impact antibody biodistribution.[3,4] Primary pathways for systemic absorption include convective transport of antibodies through lymphatic vessels into the blood, and diffusion of antibodies across blood vessels distributed near the site of injection.[5] Diffusion across vascular endothelial cells is very slow, and convection is believed to be the primary mechanism responsible for the transport of full-length antibodies from blood fluid to interstitial tissue fluids.

Other antibody formats such as antigen-binding fragment (FAb) antibodies, which have a molecular weight around 50 kDa, may offer advantages over whole antibodies (150 kDa) through more rapid onset of action because of enhanced diffusion into the interstitial space and, in patients with normal renal function, relatively rapid clearance in urine.[6] Hence, these lighter antibodies distribute more rapidly into a larger distribution volume within the body.

Tissue distribution studies of mAbs have previously been conducted with radiolabeled, nontargeted, and targeted full-length antibodies,[5–10] and a few studies have also studied distribution of antibody fragments including FAb[5,6] and multivalent single-chain variable fragment.[9] Organ-specific physiologically based pharmacokinetic (PBPK) models have previously been used for improved understanding of factors involved in antibody distribution.[8–13]

The objectives of the present study were (1) to set up a method for quantification of nonlabeled antibodies in tissues or plasma and (2) to evaluate and compare tissue distribution and define pharmacokinetic (PK) differences of full-length and FAb antibodies in tissues relevant to cardiovascular and metabolic diseases in a mouse system that has no known antigen-binding sites. We also evaluated the perfusion procedure with respect to

Abbreviations used: FAb, antigen-binding fragment; AUC, area under the curve; PD, pharmacodynamics; PK, pharmacokinetics; PBS, phosphate-buffered saline; LLOQ, low limit of quantification; T_{max}, time of maximum concentration; PBPK, physiologically based pharmacokinetic.

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manual scores of blood contamination. Additionally, we discuss the use of PBPK models in this area.

MATERIALS AND METHODS

In Vivo PK Analysis in Mice

Twenty-seven healthy C57/B6 mice (7–8 weeks old, females, Charles River, Wilmington, MA), nonfasted, with body weight of 20–22 g were used in the *in vivo* distribution PK studies of one nontargeted human IgG1antibody (NIP228 control antibody; 11.8 mg/mL) and its FAb fragment (NIP228 control FAb antibody; 13.42 mg/mL). The study was approved by Göteborg preclinical ethical committee. The mice were randomly divided into two treatment groups to receive one single (bolus) intravenous dose of 10 mg/kg of either human IgG1 or its FAb fragment via tail with the dosing volume of 10 mL/kg. Fifteen mice were injected with human IgG1, with groups of three mice sacrificed at each of the following time points: 2, 24, 48, 168, and 504 h postinjection. Additionally, 12 mice were injected with human FAb fragment, with groups of three mice sacrificed at each of the following time points: 2, 24, 48, and 168 h postinjection. The number of samples per time point is the same as used by Garg and Balthasar[8]. Mice were anaesthetized with Isoflurane (Forene®; Abbot, Scandinavia, Sweden), and the blood was collected with heart puncture. The right auricle was then cut open and 10 mL of phosphate-buffered saline (PBS; Sigma, Saint Louis, MO). PBS was perfused via the left heart ventricle before the removal of all tissues to avoid contamination from the antibodies in the circulation. For each animal sacrificed, the following tissues were harvested: pancreas, heart, aorta, brain, adipocyte tissue, liver, kidney, lung, and muscle. For the mice treated with human IgG1, the tissues were scored according to the following gradation interval: 0 = very good perfusion (no visible blood in organ); 1 = good perfusion (minor pale pink coloration of organ, no visible venules); 2 = partial perfusion (visible venules); 3 = unsuccessful perfusion (visible blood vessels). The perfused tissues were stored at –80°C pending antibody analysis.

The plasma samples were centrifuged at 2500 g for 5 min at 4°C and stored in plastic Eppendorf tubes placed on ice during handling and stored at –80°C pending quantification of the antibodies.

Homogenization of Tissues

Preweighed perfused organs were transferred to Precellys tubes (specific for type and organ, as specified in Table 1; CK14 or CK28 tubes; Bertin Technologies, Rockville, MD) and cold PBS, containing 0.5% Tween and protease inhibitor Cocktail tablets (Complete™; Roche Diagnostics, Basel, Switzerland) were added (5 µL per 1 mg tissue). Homogenization was made on ice using Precellys® 24 Homogenizer (Precellys® 24; Bertin Technologies, Rockville, MD) with specific parameters for each tissue type during handling (Table 1). Thereafter, the homogenates were transferred to Lobind tubes (Eppendorf, Hauppauge, NY), and centrifuged at 10,000 g for 1 h at 4°C. The supernatants were collected, aliquoted, and stored at –80°C pending analysis.

Table 1. Homogenization Parameters for Specific Tissues and Organs

Tissue	Tube	Specific Parameters
Brain	CK14	5000 rpm; 1 × 10 s
Adipocyte tissue	CK14	5000 rpm; 2 × 10 s
Kidney	CK14	5000 rpm; 2 × 10 s
Liver	CK14	5000 rpm; 1 × 10 s
Pancreas	CK28	5000 rpm; 1 × 30 s; 1 × 15 s
Heart	CK28	6500 rpm; 1 × 30 s; 1 × 15 s
Muscle	CK28	6000 rpm; 2 × 20 s
Lung	CK28	5000 rpm; 1 × 30 s; 1 × 15 s
Aorta	CK14	5000 rpm; 1 × 30 s; 1 × 15 s

Quantification of Human IgG1 and Its Fragment FAb in Mouse Tissue and Plasma Samples

Generic sandwich ELISA methods (with one capture antibody and one detection antibody) were qualified to measure levels of total human IgG1or total human FAb IgG1 in mouse plasma and tissues samples using the Gyrolab platform (Gyros AB, Uppsala, Sweden).

Total human IgG1 was captured by the biotinylated mouse monoclonal anti-human IgG1 antibody,100 µg/mL (clone JDC-10; Southern Biotech, Birmingham, AL), and detected by Alexa-labeled mouse monoclonal anti-human IgG1 antibody (25 nM; clone G18-145; BD Pharmingen, Franklin Lakes, NJ) on Gyrolab Bioaffy 200 CD (Gyros AB, Uppsala, Sweden). The biotin-labeled capture antibody was diluted to 100 µg/mL by filtered 0.01% PBS Tween solution. The Alexa-labeled detection antibody was centrifuged at 2500 g for 4 min, and then diluted to 25 nM in Rexxip F (Gyros AB, Uppsala, Sweden). For tissue analysis, homogenates were diluted fivefold in Rexxip A buffer (Gyros AB, Uppsala, Sweden). Calibration samples (standards and controls) were prepared in blank homogenates in exactly the same conditions as for samples. Plasma samples were diluted 100-fold in Rexxip A buffer. All samples were analyzed in duplicate. Assay accuracy and assay precision were determined by analysis of five replicates of validation control samples at three concentrations over the calibration range tested. Low limit of quantification (LLOQ) was determined from the lowest standard if it is three times above background.

The human FAb assay was performed exactly as the human IgG1 assay, but in this method human FAb was captured by the biotinylated goat anti-human F(Ab)2 / F(Ab′) (109-606-097; Jackson Immunoresearch, West Grove, PA), and detected by Alexa-labeled goat anti-human F(Ab′)2 (109-606-097, Jackson Immunoresearch, West Grove, PA) on CD1000. For tissue analysis, homogenates (standards, controls, and samples) were diluted 2–10-fold in Rexxip A buffer.

Standards, controls, unknown samples, wash solution (filtered 0.01% Tween in PBS), and capture and detection antibodies were added to a 0.2-mL 96-well PCR plate (Thermo Scientific, Waltham, MA) according to the Gyrolab method. The plate was covered with a microplate foil (Gyros AB) and centrifuged at 1500 g in 4°C for 5 min. The plates and the CD200/CD1000 were then loaded onto the Gyrolab and run with 0.01% (PBS) Tween solution in the wash 1 station along with needle wash and with 20% ethanol in the wash 2 station. The results were analyzed in the Gyrolab Evaluator software.

Data Analysis

PK parameters were determined by linear one- or two-compartment PK analysis. Measures of area under the curve (AUC), maximum concentration (C_{max}), and time of maximum