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Application of knockout mouse models to investigate the influence of FcγR on the tissue distribution and elimination of 8C2, a murine IgG1 monoclonal antibody

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ABSTRACT

The current work examines the role of Fcγ-receptors on the elimination and tissue distribution of 8C2, a model murine IgG1 monoclonal antibody. The plasma pharmacokinetics of ^{125}Iodine-labeled 8C2 were investigated in C57BL/6 control mice, FcγRI/RIII knockout mice, and FcγRIIb knockout mice, following intravenous doses of 0.04, 0.1 and 0.4 mg/kg. Plasma samples were collected and radioactivity was counted. Concentration data were analyzed with a population pharmacokinetic model. Additionally, the tissue disposition of 8C2 was investigated using whole body autoradioluminography (WBAL) and via counting excised tissues. Areas under the plasma concentration vs. time curves $AUC_{0-10\ days} \pm SD$ (nM × days) were: 12.3 ± 0.3, 12.5 ± 1.3 and 15.1 ± 1.2 at 0.04 mg/kg; 39.3 ± 2.0, 28.9 ± 2.7 and 42.0 ± 9.4 at 0.1 mg/kg; and 225 ± 19, 158 ± 19 and 204 ± 26 at 0.4 mg/kg in C57BL/6, FcγRI/RIII(−/−) and FcγRIIb(−/−) mice. Strain was not a statistically significant predictor for any of the parameters of the population model. 8C2 plasma clearance, distribution clearance, and central compartment volume were 0.00543 L/days/kg, 0.0598 L/days/kg, and 0.057 L/kg. No substantial differences in 8C2 tissue uptake were identified by analysis of excised tissues or by WBAL. In conclusion, FcγR knockout is associated with only minor effects on the plasma and tissue disposition of 8C2, a model murine IgG1 mAb.

1. Introduction

There is rapid growth in the development of therapeutic monoclonal antibodies (mAbs), with approximately 30 mAbs in current clinical use, and more than 100 in clinical development (Reichert, 2012). The pharmacokinetics (PK) of immunoglobulin G (IgG) mAbs are complex, due to the potential influence of anti-drug antibodies, interactions with the pharmacological target (i.e., in cases where target-mediated disposition occurs), and due to processing by FcRn, which protects IgG from intracellular catabolism (Ghetie and Ward, 2002; Lobo et al., 2004; Schifferli and Taylor, 1989). Although much has been learned about the determinants of monoclonal antibody disposition, several areas of uncertainty remain, including the potential influence of surface charge (Boswell et al., 2010), off-target binding (Bumbaca et al., 2011; Vugmeyster et al., 2011), and receptor-mediated transport on IgG absorption, tissue distribution, and elimination.

With respect to the potential role of transport proteins in mAb pharmacokinetics, much attention has been given to receptors that bind to the Fc-domain of IgG, which is highly conserved across all subtypes of IgG. For example, investigations with knockout mice and with engineered antibodies have provided clear demonstration that FcRn, the Fc-receptor of the neonate, is a prime determinant of IgG elimination (Akilesh et al., 2004; Datta-Mannan et al., 2007a,b; Deng et al., 2010; Junghans and Anderson, 1996). Using similar methods, FcRn has been demonstrated to be largely responsible for the high systemic bioavailability that is typically found following subcutaneous mAb dosing (Deng et al., 2012; Wang et al., 2008), and knockout mouse models have been employed to show that FcRn is not involved in mAb distribution to the brain (Garg and Balthasar, 2009).

Fc-gamma-receptors (FcγR) (e.g., FcγRI, FcγRII, FcγRIII, and FcγRIV) are well known to contribute to the biological action of IgG antibodies (Nimmerjahn et al., 2005; Nimmerjahn and Ravetch, 2008; Siberil et al., 2007; Tarasenko et al., 2007). Several therapeutic mAbs produce their effects by inducing biological responses that are mediated by FcγR, including antibody-dependent cell cytotoxicity, the release of inflammatory molecules (such as cytokines), phagocytosis of immune complexes, and enhancement of antigen

Abbreviations: mAbs, monoclonal antibodies; PK, pharmacokinetics; IgG, immunoglobulin G; FcRn, Fc-receptor of the neonate; FcγR, Fc receptors for IgG; WBAL, whole-body autoradioluminography; AUC, area under the concentration vs. time profiles; NCA, noncompartmental pharmacokinetic analysis; MVOF, mean value of objective function; VPC, visual predictive checks.

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presentation (Nimmerjahn and Ravetch, 2006, 2008; Siberil et al., 2007; Tarasenko et al., 2007).

Perhaps due to the role of FcγR in the initiation of phagocytosis of immune complexes, FcγR have been widely considered as likely contributors to the distribution and elimination of IgG (McDonagh et al., 2008; Mould and Green, 2010; Nishio et al., 2009; Tabrizi et al., 2006, 2010; Zuckier et al., 1994). However, there have been few investigations of the influence of FcγR on mAb pharmacokinetics, and work that has been published is somewhat conflicting. For example, mAb variants that were engineered for increased affinity to FcγR were shown to demonstrate substantially altered systemic pharmacokinetics (McDonagh et al., 2008; Zuckier et al., 1994). Slow clearance of infliximab in two rheumatoid arthritis patients was associated with polymorphisms in FcγRIIIB (Nishio et al., 2009); however, the significance of this association has not yet been tested (Nishio et al., 2009). Cartron et al. (2002) reported improved survival for non-Hodgkin's lymphoma patients expressing a FcγRIIIA polymorph with increased IgG affinity, following treatment with rituximab. But, retrospective analyses found no significant differences in rituximab pharmacokinetics in the high, intermediate, or low FcγRIIIA affinity polymorph groups (Dornan et al., 2010). Similarly, abrogation of the complement and FcγR binding activity of IgG2m4, a novel engineered IgG isotype, did not result in changes in serum half-life in rhesus monkeys (An et al., 2009).

To further evaluate the role of FcγR in mAb pharmacokinetics, the plasma and tissue disposition of a model IgG1 mAb, 8C2, were investigated in control (wild-type) mice and in knockout mice lacking functional FcγR expression.

2. Materials and methods

2.1. Materials

8C2, a murine IgG1 anti-topotecan antibody, was employed as a model mAb for this study. The antibody was produced and purified from the culture of hybridoma cells, as previously described (Chen and Balthasar, 2007). Sodium iodide (Na-^{125}I) was obtained from Perkin Elmer Inc. (Waltham, MA). Chloramine-T, sodium metabisulfite, calcium sulfate ($CaSO_4$), and carboxymethyl cellulose (CMC) were from Sigma Life Science (St. Louis, MO). Potassium iodide (KI) was obtained from Fisher Scientific (Pittsburgh, PA).

2.2. Animals

Three mice strains were used: (a) B6.129P2-*Fcer1g*^{tm1Rav} N12 mice, deficient in the gamma chain subunit of FcγRI and FcγRIII (FcγRI/RIII(−/−)), (b) B6.129S4-*Fcgr2b*^{tm1TtK} N12 mice, which are deficient in expression of the inhibitory receptor, FcγRIIb (FcγRIIb(−/−)), and (c) C57BL/6 wild-type (WT) mice (as a control reference strain). Of note, the C57BL/6 strain was used as the genetic background strain for each of the knockout strains used in this work. All mice were obtained from Taconic Laboratories, Hudson, NY. Animal protocols were conducted with approval from the Institutional Animal Care and Use Committee of the State University of New York at Buffalo. Animals were housed under a standard light/dark cycle, allowed free access to food and water, and maintained in an environment with controlled temperature and humidity. Mice were also kept on autoclaved KI-water (0.2 g/L) to block the thyroidal uptake of free iodine, starting 2 days prior to injection of ^{125}I-8C2.

2.3. Iodination of IgG

8C2 was radiolabeled with ^{125}I using a modified Chloramine-T method, as described in prior work (Garg and Balthasar, 2007). Briefly, 10 μL of Na-^{125}I (100 mCi/mL) was added to the antibody solution, and 20 μL of Chloramine-T (1 mg/mL in phosphate buffer) was then added to the mixture. The reaction was stopped after 90 s by the addition of 25 μL Na-metabisulfite (1 mg/mL in phosphate buffer), followed by 40 μL KI (10 mg/mL). Iodinated protein was purified by loading the reaction mixture unto a Sephadex G-25 column (GE Healthcare, NJ). The purity of the iodinated IgG was assessed using instant thin layer chromatography (PE SiL-G, Whatman Ltd., Kent, England) (Garg and Balthasar, 2007). For all experiments, the purity of the iodinated preparation was higher than 99%.

2.4. Pharmacokinetics and tissue distribution studies

2.4.1. 8C2 plasma PK

8C2 was administered intravenously at doses of 0.04, 0.1 and 0.4 mg/kg, to groups of wild-type, FcγRI/RIII(−/−), and FcγRIIb(−/−) mice (20–38 g, n = 3–4/group). The dosing solution was a mixture of the indicated 8C2 dose and a tracer dose of ^{125}I-8C2 (∼10 μCi/mouse). Blood samples (∼20–40 μL) were collected from the retro-orbital plexus or from the sub-mandibular vein at 1 h, 3 h, 8 h, and at 1, 2, 4, 7 and 10 days. Blood samples were centrifuged at 13,000 rpm for 5 min. Plasma fractions were collected and radioactivity was counted using a gamma counter (LKB Wallac 1272, Wallac, Turku, Finland). Radioactive counts were corrected for decay and background, and 8C2 plasma concentration was determined.

2.4.2. 8C2 tissue distribution

8C2, at a dose of 8 mg/kg plus a tracer quantity of ^{125}I-8C2 (∼400 μCi/kg ^{125}I activity, ∼10 μCi/mouse) was injected intravenously to C57BL/6, FcγRI/RIII(−/−), and FcγRIIb(−/−) mice (20–25 g). Samples of blood, spleen, kidney, liver, heart, lung, thymus, gastrointestinal tract (GI), muscle, bone, fat and skin were harvested at 1 h, 2 h, 6 h, 12 h, 1, 2, 4, 7 and 10 days post dosing (n = 3 mice/time point/strain). Collected tissues were blotted dry, weighed, and counted for radioactivity. Radioactive counts were decay and background corrected, and 8C2 concentration was determined. The densities of all tissues were assumed to be 1 g/mL.

2.4.3. Whole-body autoradioluminography (WBAL)

Two days post injection of 8 mg/kg ^{125}I-8C2 to C57BL/6, FcγRI/RIII(−/−), and FcγRIIb(−/−) mice, one mouse from each strain was euthanized. Carcasses were immediately frozen in a hexane/dry ice bath (∼−75 °C) and embedded in 2.5% carboxymethyl cellulose. After being completely frozen, sagittal plane sectioning was performed using a Leica CM3600 cryomicrotome (Leica Microsystems Inc., Bannockburn, IL). Sections of 50 μm thick were collected at different sagittal planes using adhesive Scotch tape (3 M, St. Paul, MN). For anatomical identification of exposed organs, true colored images were also taken. Collected sections were freeze dehydrated at −20 °C for 3 days. Sections were then equilibrated to room temperature in a $CaSO_4$ desiccator. Selected sections were then exposed to phosphor imaging plates (BAS-SR2025, FUJIFILM Medical Systems) for 3 days. Imaging plates were scanned using bio-imaging analyzer system (BAS-5000, FUJIFILM). The image reader, version 1.8, and the image analyzer, Multi Gauge, version 3 (FUJIFILM), was used for reading and analyzing the image.

2.5. Noncompartmental pharmacokinetic data analysis

Noncompartmental pharmacokinetic analysis (NCA) (WinNonlin 6.1, Phoenix, Pharsight Corporation, Palo Alto, CA) was used to calculate mean areas under the concentration vs. time curves ($AUC_{0-10\ days}$). Mean 8C2 blood and tissues concentration vs. time