Pharmacokinetic and Pharmacodynamic Effects of High-Dose Monoclonal Antibody Therapy in a Rat Model of Immune Thrombocytopenia

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Abstract

Intravenous administration of pooled, polyvalent human immunoglobulin (IVIG) has been used for over 20 years as a therapy for immune thrombocytopenia (ITP). IVIG is available in limited quantities, and clinical preparations have been associated with the transfer of human pathogens. We have proposed that high-dose monoclonal antibody may be used in lieu of IVIG to achieve beneficial effects in the treatment of ITP. The current study investigates the effects of high-dose monoclonal antibody therapy in a rat model of ITP. Hybridoma cells secreting a murine monoclonal antiplatelet antibody (7E3) and murine monoclonal anti-methotrexate IgG (AMI) were grown in serum-free media. Next, 7E3, 8 mg kg^{-1}, was administered intravenously to rats following pretreatment with saline or AMI (1 g kg^{-1} IV). AMI and 7E3 plasma concentrations were determined via enzyme-linked immunosorbent assay, and platelet count was determined with a Cell-Dyne hematology analyzer. Severe, transient thrombocytopenia was induced by 7E3. Platelet counts dropped to ~8% of initial values within 1 hour after 7E3 administration. AMI pretreatment dramatically affected 7E3-induced thrombocytopenia, significantly altering the time course of thrombocytopenia ($P < .05$) and significantly decreasing the severity of 7E3-induced thrombocytopenia (ie, following AMI pretreatment, nadir platelet count was greater than 8-fold that of the control group, $P < .05$). In addition, AMI pretreatment induced a 57% increase in 7E3 clearance (1.13 ± 0.13 mL h^{-1} kg^{-1} vs 0.72 ± 0.08 mL h^{-1} kg^{-1}, $P < .05$). Consequently, high-dose monoclonal antibody therapy attenuated thrombocytopenia and produced a moderate increase in the clearance of antiplatelet antibodies in a rat model of ITP.

Keywords: Immune thrombocytopenia, autoimmune, IVIG, autoantibody

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Introduction

Immune thrombocytopenic purpura is a common hematological disorder characterized by low platelet count and mucocutaneous bleeding. Immune thrombocytopenia (ITP) patients develop autoantibodies that bind to platelet surface proteins, and the opsonized platelets are rapidly eliminated by cells of the reticuloendothelial system. Standard therapies for ITP, including corticosteroids and splenectomy, increase platelet counts above critical levels in the majority of treated patients. However, ~35% of ITP patients are refractory to these therapies, and these patients are at high risk for fatal hemorrhage.[1]

High-dose intravenous immunoglobulin (IVIG) was introduced as a treatment of ITP by Imbach et al in 1981. They reported that IV administration of high-dose pooled human gamma immunoglobulins increased platelet counts in pediatric ITP patients.[2] Thereafter, several clinical studies confirmed that IVIG could produce dramatic increases in platelet counts in ITP.[3] Although this therapy is very effective in treating ITP, IVIG is extremely expensive[4] and IVIG administration has been associated with the transfer of human pathogens.[5] Additionally, IVIG is a human blood product that is available in only limited quantities.[6]

Since the introduction of IVIG therapy for ITP, several mechanisms have been proposed to account for IVIG effects. These mechanisms may be generally categorized as (1) requiring engagement of the Fc domains of IVIG, (2) requiring engagement of the Fab domains of IVIG, or (3) requiring engagement of both the Fab and the Fc domains of IVIG. For example, it has been proposed that IgG monomers contained within the IVIG preparation may lead to the direct, competitive inhibition of Fcγ-receptors or FcRn. Inhibition of Fcγ-receptors may lead to a decreased rate of Fcγ-receptor–mediated destruction of antibody-opsonized platelets.[7] Alternatively, competitive inhibition of FcRn may lead to an increase in the rate of elimination of pathogenic IgG autoantibodies.[8] In each case, IVIG effects would require engagement of the Fc domains but not the Fab domains. On the other hand, it has been proposed that IVIG effects are due to the neutralization of pathogenic antibodies by anti-idiotypic antibodies or due to immunomodulation mediated by anticytokine antibodies.[9] These effects would require engagement of the Fab domains of IVIG but not the Fc domains. Several proposed mechanisms would

require engagement of both Fab and Fc domains. Included in this group is the Salama hypothesis that IVIG contains anti–red cell antibodies, which opsonize red cells upon IVIG administration; in this hypothesis, IVIG effects involve indirect Fcγ-receptor blockade, mediated by antibody-coated red cells.[10] Additionally, it has been proposed that anti-idiotypic antibodies may cross-link the B-cell receptor and FcγRIIb on autoantibody-producing cells, leading to apoptosis and decreases in autoantibody production.[11]

The requirement of Fab and Fc domains for IVIG effects has been investigated in a few small clinical studies. For example, Tovo et al reported that Gammavenin, a partially digested IVIG preparation that primarily consists of Fab_2 fragments, increased platelet counts in some chronic ITP patients. These investigators commented that "the effectiveness of [IVIG] in ITP is not necessarily dependent on the Fc fragment" and their results suggest that IVIG effects may be mediated, in part, via Fab domains.[12] On the other hand, Debre et al reported that infusion of Fc gamma fragments led to a rapid increase in platelet counts in 11 out of 12 pediatric patients with acute ITP.[13] Because of the small scale of these studies and the possible presence of contaminating substances (eg, cytokines, soluble cytokine receptors, undesired Fc fragments), the relative importance of Fab and Fc domains for IVIG effects remains uncertain.

We hypothesize that the majority of benefit derived from IVIG therapy is mediated via mechanisms exclusively dependent on the Fc domain (eg, FcRn inhibition), where the specificity of Fab domains is irrelevant. As such, we have hypothesized that high-dose monoclonal antibody therapy may be used, in lieu of IVIG, to increase the elimination of pathogenic antiplatelet antibodies and to attenuate thrombocytopenia in ITP. The present study tests these hypotheses by investigating the pharmacokinetic and pharmacodynamic effects of high-dose monoclonal antibody therapy in a rat model of ITP.

Design and Methods

Reagents

Monoclonal AMI and monoclonal antiplatelet antibody (7E3) were produced and purified in our laboratory.[14,15] Human glycoprotein (GP) IIb/IIIa was purchased from Enzyme Research Laboratories (South Bend, IN). Bovine serum albumin (BSA), goat antimouse IgG-alkaline phosphatase conjugate (Fab-specific), and buffer reagents were purchased from Sigma (St Louis, MO). p-Nitrophenyl phosphate was purchased from Pierce (Rockford, IL). BSA-methotrexate (BSA-MTX) was prepared, using the method published previously, in our laboratory.[14] Outdated human platelets were obtained from the American Red Cross (Buffalo, NY).

Animals

Female Sprague-Dawley rats, 200 to 225 g, were obtained from Harlan (Indianapolis, IN) and housed in the university animal facility. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University at Buffalo.

AMI and 7E3 Enzyme-Linked Immunosorbent Assay Procedure

AMI plasma concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) method.[16] Briefly, Nunc Maxisorp 96-well microplates (Nunc model 4–42404, Roskilde, Denmark) were incubated with BSA-MTX conjugate (0.25 mL/well) at 4°C overnight. Plasma samples and AMI standards (0.25 mL/well) were added, and the plates were incubated at room temperature for 2 hours. The plates were then incubated with goat–antimouse antibody-alkaline phosphate conjugate (diluted 1:500 in phosphate buffer (PB), 0.25 mL/well) for 1 hour at room temperature. Finally, p-nitrophenyl phosphate (4 mg mL^{-1} in diethanolamine buffer, pH 9.8, 0.2 mL/well) was added and the change of absorbance with time at 405 nm was monitored by a microplate reader (Spectra Max 250, Molecular Devices, Sunnyvale, CA). Between each step, the plates were washed with PB-0.05% Tween-20 buffer 3 times and with double-distilled water 3 times. Standard curves were linear in the concentration range of 25 to 250 ng mL^{-1}. The ELISA method for measuring 7E3 plasma concentration was reported elsewhere.[15]

AMI Pharmacokinetics

The disposition of AMI was assessed following IV doses ranging from 1 to 1000 mg kg^{-1} to test the hypothesis that AMI clearance will increase with increasing dose (ie, consistent with the capacity-limited nature of FcRn transport). Prior to AMI administration, animals were anesthetized with ketamine/xylazine (75/15 mg kg^{-1}) and then instrumented with a jugular vein cannula. Two or 3 days later, AMI was administered intravenously via the jugular vein cannula at doses of 1, 5, 10, 50, 100, 500, or 1000 mg kg^{-1} (3 rats for each group). Blood samples (0.2 mL) were obtained from the jugular vein cannula at 0, 1, 3, 6, 12, 24, 48, 96, and 168 hours and transferred to heparinized microcentrifuge tubes. Blood was then centrifuged at 12 000 rpm for 5 minutes and plasma was stored at –20°C. Plasma AMI concentrations were determined via ELISA.

AMI plasma concentration versus time data were analyzed using standard noncompartmental techniques. AMI doses were given by rapid IV injection (1, 5, 10, 50 mg kg^{-1}) or by short-term infusion over 5 to 30 minutes (100, 500, and 1000 mg kg^{-1}). The terminal half-life ($t_{1/2}$) was determined

from the equation $t_{1/2} = 0.693/\lambda$, where λ is the negative ln-linear slope of the terminal phase of the AMI concentration-time profile. The terminal phase was determined from the last 3 to 4 time points for each curve. The area under the concentration-time curve (AUC) and the area under the first moment curve (AUMC) from time zero to infinity were calculated using the log-trapezoidal rule. Clearance (CL) was determined as the quotient of the AMI dose and the AUC. Mean residence time of disposition (MRT_d) was calculated from the equation $MRT_d = AUMC/AUC - T/2$, where T refers to the duration of AMI infusion. The volume of AMI distribution at steady state (V_{ss}) was calculated as the product of MRT_d and CL.

AMI Effects on 7E3-Platelet Binding

The effect of AMI on 7E3-platelet binding was investigated in vitro. Human platelets (5.5×10^8/mL) were incubated with 7E3 (5-75 μg/mL) in the presence or absence of AMI (20 mg/mL) for 2 hours. Samples were then centrifuged at 2000 g for 5 minutes. The supernatant was obtained, and 7E3 concentration in the supernatant was determined by ELISA. The affinity (K_a) and capacity (R_t) of 7E3-platelet binding were assessed through the use of the following equation, which assumes equilibrium binding conditions:

$$F_f = \frac{7E3_f \times K_a + 1}{1 + K_a \times 7E3_f + K_a \times R_t} \quad (1)$$

where F_f represents the free fraction of 7E3, $7E3_f$ is the molar concentration of unbound 7E3 (ie, the assayed concentration of 7E3 in the supernatant), K_a is the equilibrium association constant, and R_t is the concentration of binding sites for 7E3.

Pharmacokinetic and Pharmacodynamic Effects of AMI in the Rat Model of ITP

The effect of high-dose AMI was investigated in the passive rat ITP model.[8,15] Three days prior to treatments, animals were anesthetized with ketamine/xylazine (75/15 mg kg^{-1}) and instrumented with jugular vein cannulas. Rats were pretreated with either saline or an IV dose of AMI at 1 g kg^{-1} (n = 4/group) and then challenged with an IV bolus dose of a murine monoclonal antibody against GPIIb/IIIa (7E3, 8 mg kg^{-1}). Blood samples were taken from the jugular vein cannula at 0, 1, 3, 6, 12, 24, 48, 96, and 168 hours. Blood platelet counts were measured up to 24 hours after 7E3 treatment using a Cell-Dyne 1700 cell analyzer. Plasma 7E3 and AMI concentrations were measured by ELISA. The platelet count nadir for each animal was defined as the lowest observed platelet count. Because of substantial interanimal variability in the pretreatment platelet count, data were normalized by the initial platelet counts for each animal to facilitate the comparison of data obtained from different animals. Noncompartmental analyses were performed to determine the CL, terminal half-life ($t_{1/2}$), and V_{ss}.

Statistical Analysis

Noncompartmental analyses for AMI and 7E3 pharmacokinetics were performed using Winnolin software (version 2.1, Pharsight Corp, Apex, NC). Differences in CL, V_{ss}, and $t_{1/2}$ between all AMI dose groups were analyzed by 1-way analysis of variance (ANOVA; $\alpha = 0.05$). In vitro binding data were fitted using ADAPT II (release 4, Biomedical Simulations Resource, University of Southern California, Los Angeles, CA). Differences in the time course of platelet counts between saline control and AMI-treated groups were tested by 2-way, repeated measures ANOVA ($\alpha = 0.05$). Differences of platelet nadir values and 7E3 clearance values between control and AMI-treated groups were analyzed by Mann-Whitney nonparametric t tests ($\alpha = 0.05$), and comparisons of the dose-normalized plasma exposure of AMI were performed with 1-way ANOVA, using the Dunnett posttest. All statistical analyses were performed using Prism Software (GraphPad Software, Inc, San Diego, CA).

RESULTS

AMI Pharmacokinetics

AMI concentration vs time data are plotted in Figure 1. AMI plasma concentrations declined in a biexponential fashion. Table 1 lists the value of CL, V_{ss}, MRT_d, and $t_{1/2}$ obtained from noncompartmental analyses of the AMI pharmacokinetic data. To assess possible dose dependencies in AMI

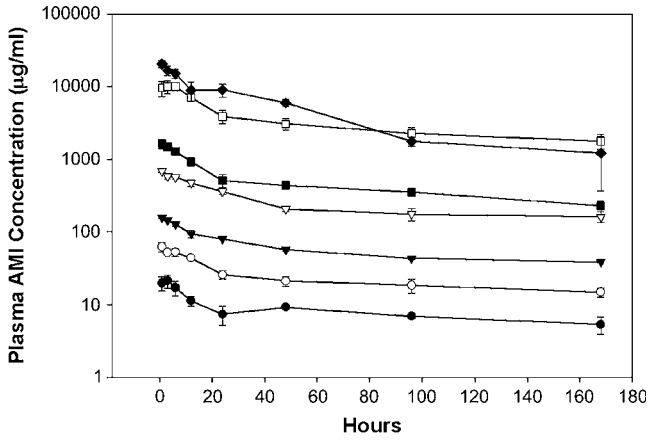

Figure 1. Plasma AMI pharmacokinetic profile. Data are plotted on a semilogarithmic scale. Rats (n = 3/group) received 7 IV doses of AMI: 1 (●), 5 (○), 10 (▼), 50 (▽), 100 (■), 500 (□), and 1000 (♦) mg kg^{-1}. AMI concentrations were determined via ELISA. Error bars represent the standard deviation of the mean concentration at each time point. The clearance of AMI increased with dose, from 0.39 ± 0.13 mL h^{-1} kg^{-1} (1 mg kg^{-1}) to 1.19 ± 0.19 mL h^{-1} kg^{-1} (1 g kg^{-1}), calculated from the concentration versus time profiles. AMI indicates anti-methotrexate IgG; ELISA, enzyme-linked immunosorbent assay; IV, intravenous.

Table 1. Pharmacokinetic Parameters of Anti-methotrexate IgG From Noncompartmental Analysis*

Dose (mg kg^{-1})	$t_{1/2}$ (h) †	CL (mL h^{-1} kg^{-1}) †	V_{ss} (mL kg^{-1})†	MRT_d (h)†
1	172 ± 88	0.39 ± 0.13	84.2 ± 14	247 ± 129
5	232 ± 2.2	0.59 ± 0.07	185 ± 21	316 ± 3.25
10	242 ± 35	0.46 ± 0.04	140 ± 11	308 ± 49.0
50	309 ± 11	0.46 ± 0.14	182 ± 17	424 ± 158
100	129 ± 30	0.86 ± 0.14	143 ± 17	169 ± 41.1
500	115 ± 73	0.63 ± 0.09	98.1 ± 29.7	161 ± 75.9
1000	59.0 ± 30	1.19 ± 0.19	88.7 ± 28.7	74.6 ± 30.5

*Please note that the parameter values reported in this table should be considered as rough estimates. V_{ss} and MRT_d have been estimated with standard noncompartmental analyses that assume linear pharmacokinetics. Additionally, accurate assessment of the terminal half-life of anti-methotrexate IgG in plasma is not possible because of the limited duration of sampling (168 hours). $t_{1/2}$ indicates terminal half-life; CL, clearance; V_{ss}, volume at steady state; MRT_d, mean residence time of disposition.
†1-way analysis of variance, $P < .005$ (n = 3/group).

pharmacokinetics, the quotients of the area under the AMI plasma concentration versus time curve and AMI dose (ie, AUC/AMI dose) were compared. The data were found to be significantly different ($P = .002$), with a trend of decreasing values of dose-normalized AMI plasma exposure with increasing doses of AMI (Figure 2). In comparison to the AUC/AMI dose ratio observed for the 1 mg/kg AMI dose, significant differences in the dose-normalized AUC were found for AMI doses of 5 mg/kg ($P < .05$), 100 mg/kg ($P < .01$), 500 mg/kg ($P < .05$), and 1000 mg/kg ($P < .001$). Consistent with the hypothesis that high-dose monoclonal antibody therapy would saturate FcRn (which protects IgG from intracellular catabolism), the clearance of AMI increased with dose, from 0.39 ± 0.13 mL h^{-1} kg^{-1} (1 mg kg^{-1}) to 1.19 ± 0.19 mL h^{-1} kg^{-1} (1 g kg^{-1}). In contrast, $t_{1/2}$ decreased with dose, from 171.9 ± 87.8 hours (1 mg kg^{-1}) to 59 ± 30.4 hours (1 g kg^{-1}). Because of this dose dependency, the reported CL values should be considered as time- and concentration-averaged values. In addition, the methods used to calculate V_{ss} and MRT_d assume linear pharmacokinetics; therefore, the reported V_{ss} values should be considered as rough estimates. Based on the 1-way ANOVA analysis, significant differences for CL, V_{ss}, MRT_d, and $t_{1/2}$ values between different doses were determined ($P < .005$).

AMI Effects on 7E3-Platelet Binding

As shown in Figure 3, AMI did not alter 7E3-platelet binding. Computer-fit values of K_a and R_t were 6.9 ± 1.7 × 10^8 M^{-1} and 1.1 ± 0.1 × 10^{-7} M, respectively, when 7E3 was incubated with platelets in the presence of AMI, and 7.6 ± 1.5 × 10^8 M^{-1} and 1.1 ± 0.1 × 10^{-7} M, respectively, when 7E3 was incubated with platelets without AMI ($P > .05$ for K_a and R_t). This finding is consistent with the expectation that AMI, an anti-methotrexate antibody, would not alter the interaction of the 7E3 idiotype with 7E3 binding sites on platelets.

AMI Effects on 7E3-Mediated Thrombocytopenia and 7E3 Pharmacokinetics

Platelet count versus time profiles for the control and AMI-treated groups are shown in Figure 4. Severe thrombocytopenia was induced by 7E3 in animals pretreated with saline. Platelet counts reached nadir values within 1 to 3 hours after 7E3 administration; nadir counts were 8.1 ± 3.8% of initial platelet counts. AMI pretreatment at 1 g kg^{-1}

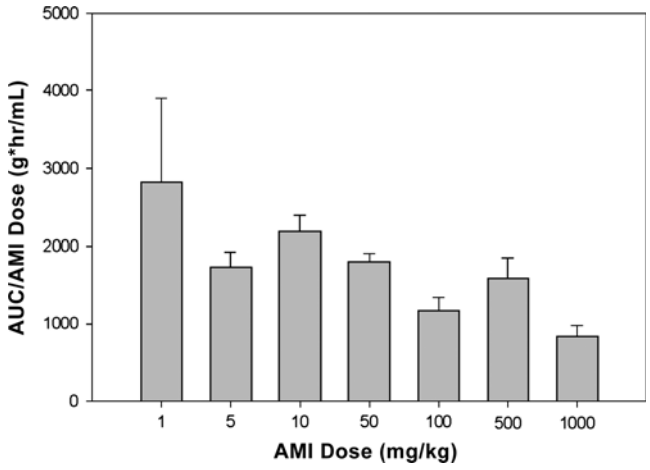

Figure 2. Dose-normalized plasma exposure of AMI. The dose-normalized plasma exposure of AMI was calculated as the quotient of the AUC and the AMI dose, for each AMI dose investigated. The data show a general trend toward a decrease in AUC/AMI dose with increasing AMI dose, which is consistent with an increase in AMI clearance with increasing AMI dose. The data were evaluated by 1-way ANOVA and found to be significantly different ($P = .002$). Subsequent analysis with the Dunnett posttest, using the AUC/AMI dose ratio observed following 1 mg/kg AMI as the reference value, indicated significant differences following AMI doses of 5 mg/kg ($P < .05$), 100 mg/kg ($P < .01$), 500 mg/kg ($P < .05$), and 1000 mg/kg ($P < .001$). AMI indicates anti-methotrexate IgG; ANOVA, analysis of variance; AUC, area under the AMI plasma concentration versus time curve.

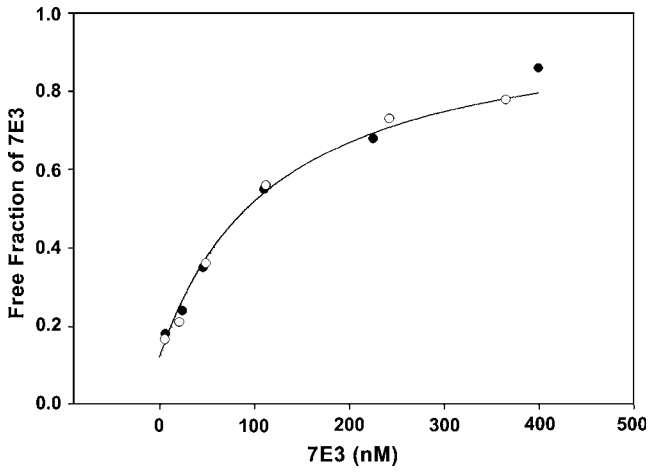

Figure 3. AMI effects on 7E3-platelet binding. Platelets were incubated with 7E3 (5-75 µg/mL) in the absence (○) or in the presence (•) of AMI (20 mg/mL). Binding data were fitted as described in the text; best fit lines for data collected in the absence and presence of AMI are plotted as solid and dashed lines, respectively. As shown, the lines are essentially superimposed; the fitted parameters, K_a and R_t, did not differ significantly. AMI indicates anti-methotrexate IgG.

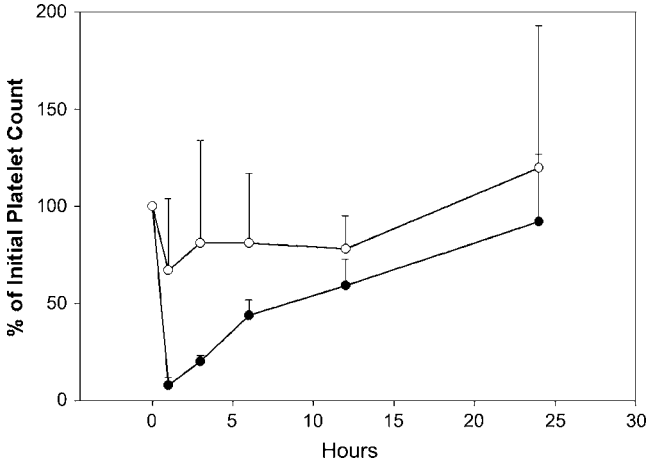

Figure 4. AMI effects on the time course of 7E3-induced thrombocytopenia. Rats received 1 g kg^{-1} AMI or saline, followed by 8 mg kg^{-1} 7E3. AMI and 7E3 were given intravenously, and platelet counts were analyzed by a Cell-Dyne 1700 multiparameter hematology analyzer. Symbols represent average percent of initial platelet count data for the saline-treated group (•) and the AMI-treated group (○) (n = 4/group). Error bars represent the standard deviation of the mean percent platelet count at each time point. AMI treatment altered the time course of thrombocytopenia significantly (as assessed by 2-way, repeated measures ANOVA, $P < .05$). Nadir platelet counts in the AMI-treated group were 8-fold greater than those observed for the control animals ($P = 0.028$, nonparametric *t* test). AMI indicates anti-methotrexate IgG; ANOVA, analysis of variance.

significantly altered the platelet count versus time profile ($P < .05$), and the platelet nadir in the AMI-treated group was 8-fold that observed in control animals (66.9 ± 38.4% vs 8.1 ± 3.8% of initial platelet count, $P = 0.028$). The 7E3 pharmacokinetic profiles for both the control group and the treated group are shown in Figure 5. AMI pretreatment increased the 7E3 clearance from 0.72 ± 0.08 mL h^{-1} kg^{-1} to 1.13 ± 0.13 mL h^{-1} kg^{-1} ($P = 0.028$). Calculated values for CL, V_{ss}, and $t_{1/2}$ are summarized in Table 2.

DISCUSSION

In 1951, Harrington et al demonstrated that healthy volunteers developed thrombocytopenia following infusion of plasma obtained from ITP patients.[17] Subsequent work demonstrated similarities between the plasma factors associated with ITP and immunoglobulins, thus supporting the hypothesis that ITP is mediated by autoantibodies.[18] Several platelet membrane targets for autoantibodies have been identified, including GPIIb/IIIa and GPIb/IX, and it is currently accepted that thrombocytopenia in ITP is mediated by autoantibody-platelet binding.[19-22] Based on the monotonic nature of antibody-antigen binding, it is highly likely that platelet opsonization increases as a direct function of increasing concentrations of autoantibody in plasma. Consequently, it is not surprising that therapies that reduce autoantibody concentrations in plasma (eg, plasmapheresis, protein-A immunoadsorption) palliate thrombocytopenia in many ITP patients.[23,24]

More than 40 years ago, Brambell et al reported that the fractional catabolic rate (ie, elimination rate constant) of IgG increased with increasing concentrations of IgG in serum.[25] Additionally, Brambell et al proposed that this observation may be explained by the presence of a receptor-mediated transport system that protects IgG from catabolism. The authors proposed that the transport system may be saturated at high serum concentrations of IgG, leading to increases in the fractional catabolic rate. This transport system was later demonstrated to be present in rat epithelium,[26] and the receptor was identified, cloned, and named FcRn (ie, the neonatal Fc-receptor).[27] In 1996, 3 groups independently found that the clearance of IgG is 10-fold greater in FcRn-knockout mice than in controls, supporting the hypothesis that FcRn protects IgG from catabolism.[28-30]

Hansen and Balthasar found that IVIG accelerated the elimination of an antiplatelet antibody (7E3) in a rat ITP model and hypothesized that this observation resulted from IVIG-mediated saturation of FcRn.[8] Additionally, Hansen and Balthasar demonstrated that IVIG increased 7E3 clearance in control mice (which is similar to the results found in rats) but did not increase 7E3 clearance in FcRn-knockout mice (which is consistent with the hypothesis that IVIG increased the clearance of the pathogenic antiplatelet antibody via saturation of FcRn).[31] Mechanism-based mathematical modeling suggested that 50 ± 11% of the overall effect of IVIG on 7E3-induced thrombocytopenia was due to the

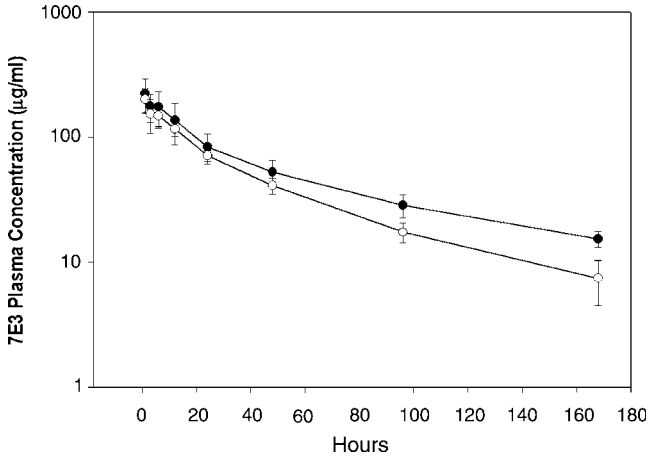

Figure 5. Plasma 7E3 pharmacokinetic profile following AMI pretreatment. Data are plotted on a semilogarithmic scale. Rats (n = 4/group) received AMI (1 g kg^{-1}) or saline intravenously followed by 7E3 (8 mg kg^{-1}). The 7E3 concentrations were determined via ELISA. Symbols represent the average 7E3 plasma concentration data of the saline-treated group (•) and the AMI-treated group (○). Error bars represent the standard deviation of the mean concentration at each time point. AMI treatment significantly increased the clearance of 7E3 ($P = 0.028$). AMI indicates anti-methotrexate IgG; ELISA, enzyme-linked immunosorbent assay.

effect of IVIG on 7E3 disposition.[32] In addition to demonstrating the importance of FcRn-inhibition in the context of the overall effects achieved by IVIG, these studies support the hypothesis that IVIG primarily achieves effects by mechanisms that are solely dependent on the immunoglobulin Fc-region.[33] Therefore, new therapies that are designed to mimic the feature of the Fc portion of IVIG could function as alternatives to IVIG, perhaps even leading to better therapeutic outcomes.

Monoclonal antibodies may serve as such alternatives. Bearing an Fc region that is highly conserved among mammals, monoclonal antibodies may block Fcγ-receptor–mediated phagocytosis of opsonized platelets and may also inhibit FcRn, increasing the elimination of pathologic antiplatelet antibodies. AMI is a murine monoclonal antibody against methotrexate.[14] Therefore, it is unlikely that the Fab regions of AMI would produce any effects that are related to the Fab regions of IVIG. Additionally, since AMI is developed from hybridoma cell culture, it may be produced in large quantities and is free from blood-borne human pathogens. AMI is a murine IgG1 antibody, and this isotype has shown high affinity to rat FcRn.[34] For all these reasons, AMI appeared to be a suitable candidate to test our hypotheses.

Table 2. Effects of Anti-methotrexate IgG on 7E3 Pharmacokinetics*

Parameters	Control	Treatment
$t_{1/2}$ (h)	118 ± 90	54.4 ± 16
CL (mL h^{-1} kg^{-1})†	0.72 ± 0.08	1.13 ± 0.14
V_{ss} (mL kg^{-1})	92.2 ± 69	65.3 ± 13.5

*CL indicates clearance; $t_{1/2}$, terminal half-life; V_{ss}, volume at steady state.
†Nonparametric *t* test, $P = 0.028$ (n = 4 for each group).

In the present study, pretreatment of 1 g kg^{-1} AMI led to a 57% increase in 7E3 clearance, which is consistent with our hypothesis that high-dose monoclonal antibody could increase 7E3 elimination by saturating the FcRn receptor. As suggested by the known specificity of AMI, and as demonstrated by the lack of an effect of AMI on 7E3-platelet binding in vitro (Figure 3), anti-idiotypic effects may be ruled out. Comparing the present data to the results of previous studies conducted with IVIG therapy at the same dose level in the same rat model, AMI induced a lower increase in the clearance of the antiplatelet antibody (ie, 57% increase vs 76% increase). The present study was not designed to compare IVIG and AMI effects, and the statistical significance of this difference is uncertain. However, if the difference is real, it may be partly explained by the known difference of binding affinity of rat FcRn to human and mouse IgG. The dissociation constants of human polyclonal IgG and mouse IgG1 to rat FcRn are 15 and 49 nM, respectively.[34] Therefore, it is possible that IVIG could produce tighter binding to rat FcRn, leading to stronger inhibition.

The impact of AMI on 7E3 disposition was consistent with the dose-dependent clearance of AMI. That is, investigations of AMI pharmacokinetics following doses ranging from 1 mg kg^{-1} to 1 g kg^{-1} demonstrated that AMI clearance increased with dose. To our knowledge, this is the first demonstration of dose-dependent increases in the elimination of monoclonal antibody. This finding, however, is consistent with the known increase in the fractional catabolic rate of IgG with increasing serum concentrations of IgG, and with the current conceptual understanding of FcRn-mediated protection of IgG.[25,35]

As shown in Table 2, AMI pretreatment was not associated with significant alterations in 7E3 volume of distribution or half-life. However, the data suggest a trend toward a decreased V_{ss} and a decreased $t_{1/2}$ of 7E3 in animals pretreated with AMI. This trend is consistent with the expectation that high-dose AMI would competitively inhibit 7E3 binding to FcRn, which would be expected to decrease the 7E3 distribution volume and increase the rate of 7E3 elimination. The failure to observe a significant alteration in V_{ss} may be due to the relatively large degree of variability in the data, the relatively small sample sizes used in this study, and potentially, to a small role of FcRn binding in the apparent volume of IgG distribution (eg, high-affinity,

low-capacity binding). The failure to observe a significant alteration in the terminal half-life of 7E3 disposition may be due to a transient effect of AMI on FcRn, where competitive inhibition of FcRn occurs primarily during the first 24 to 48 hours following AMI administration, when AMI concentrations are very high.

AMI pretreatment led to pronounced attenuation of 7E3-induced thrombocytopenia. When compared with the effects observed in this rat model following IVIG treatment, treatment with AMI appears to produce a greater degree of protection against thrombocytopenia. For example, following 1 g kg^{-1} IVIG or AMI, IVIG-treated animals demonstrated nadir platelet counts that were ~3-fold those observed in saline-treated controls, whereas AMI-treated (1 g kg^{-1}) animals exhibited nadir counts that were ~8-fold greater than those of the control group.[8] The present study was not designed to compare AMI and IVIG; however, the differences between AMI and IVIG effects, if real, may indicate that AMI is capable of more efficient blockage of Fcγ-receptors.

In conclusion, high-dose AMI monoclonal antibody therapy attenuated antiplatelet-antibody-induced thrombocytopenia and produced a moderate increase in antiplatelet antibody clearance in a rat model of ITP. To our knowledge, this is the first demonstration that high-dose monoclonal antibody therapy could increase the clearance of pathogenic antibodies in any animal model of humoral autoimmune disease. Although only 1 monoclonal antibody (AMI) was evaluated in this work, other monoclonal IgG antibodies might produce similar effects. Consequently, the results of this study suggest that high-dose monoclonal antibody may have some potential in treating ITP.

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REFERENCES

1. George JN, Woolf SH, Raskob GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood*. 1996;88:3-40.

2. Imbach P, Barandun S, d'Apuzzo V, et al. High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet*. 1981;1:1228-1231.

3. Bussel JB, Hilgartner MW. Intravenous immunoglobulin therapy of idiopathic thrombocytopenic purpura in childhood and adolescence. *Hematol Oncol Clin North Am*. 1987;1:465-482.

4. Adams JR, Nathan DP, Bennett CL. Pharmacoeconomics of therapy for ITP: steroids, i.v.Ig, anti-D, and splenectomy. *Blood Rev*. 2002;16:65-67.

5. Gomperts ED. Gammagard and reported hepatitis C virus episodes. *Clin Ther*. 1996;18:3-8.

6. Milgrom H. Shortage of intravenous immunoglobulin. *Ann Allergy Asthma Immunol*. 1998;81:97-100.

7. Fehr J, Hofmann V, Kappeler U. Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gamma globulin. *N Engl J Med*. 1982;306:1254-1258.

8. Hansen RJ, Balthasar JP. Effects of intravenous immunoglobulin on platelet count and antiplatelet antibody disposition in a rat model of immune thrombocytopenia. *Blood*. 2002;100:2087-2093.

9. Berchtold P, Dale GL, Tani P, McMillan R. Inhibition of autoantibody binding to platelet glycoprotein IIb/IIIa by anti-idiotypic antibodies in intravenous gammaglobulin. *Blood*. 1989;74:2414-2417.

10. Salama A, Mueller-Eckhardt C, Kiefel V. Effect of intravenous immunoglobulin in immune thrombocytopenia. *Lancet*. 1983;2:193-195.

11. Ballow M. Mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory diseases. *J Allergy Clin Immunol*. 1997;100:151-157.

12. Tovo PA, Miniero R, Fiandino G, Saracco P, Messina M. Fc-depleted vs intact intravenous immunoglobulin in chronic ITP. *J Pediatr*. 1984;105:676-677.

13. Debre M, Bonnet MC, Fridman WH, et al. Infusion of Fc gamma fragments for treatment of children with acute immune thrombocytopenic purpura. *Lancet*. 1993;342:945-949.

14. Lobo ED, Soda DM, Balthasar JP. Application of pharmacokinetic-pharmacodynamic modeling to predict the kinetic and dynamic effects of anti-methotrexate antibodies in mice. *J Pharm Sci*. 2003;92:1665-1676.

15. Hansen RJ, Balthasar JP. Pharmacokinetics, pharmacodynamics, and platelet binding of an anti-glycoprotein IIb/IIIa monoclonal antibody (7E3) in the rat: a quantitative rat model of immune thrombocytopenic purpura. *J Pharmacol Exp Ther*. 2001;298:165-171.

16. Tayab ZR, Balthasar JP. Development and validation of enzyme-linked immunosorbent assay for quantification of anti-methotrexate IgG and Fab in mouse and rat plasma. *J Immunoassay Immunochem*. 2004;25:335-344.

17. Harrington WJ, Minnich V, Hollingsworth JW, Moore CV. Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J Lab Clin Med*. 1951;38:1-10.

18. Shulman NR, Marder VJ, Weinrach RS. Similarities between known antiplatelet antibodies and the factor responsible for thrombocytopenia in idiopathic purpura: physiologic, serologic and isotopic studies. *Ann N Y Acad Sci*. 1965;124:499-542.

19. van Leeuwen EF, van der Ven JT, Engelfriet CP. von dem Borne AE. Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood*. 1982;59:23-26.

20. Woods VL Jr, Kurata Y, Montgomery RR, et al. Autoantibodies against platelet glycoprotein Ib in patients with chronic immune thrombocytopenic purpura. *Blood*. 1984;64:156-160.

21. Fujisawa K, Tani P, McMillan R. Platelet-associated antibody to glycoprotein IIb/IIIa from chronic immune thrombocytopenic purpura patients often binds to divalent cation-dependent antigens. *Blood*. 1993;81:1284-1289.

22. Hou M, Stockelberg D, Kutti J, Wadenvik H. Immunoglobulins targeting both GPIIb/IIIa and GPIb/IX in chronic idiopathic thrombocytopenic purpura (ITP): evidence for at least two different IgG antibodies. *Br J Haematol*. 1997;98:64-67.

23. Snyder HW Jr, Cochran SK Jr, Balint JP Jr, et al. Experience with protein A-immunoadsorption in treatment-resistant adult immune thrombocytopenic purpura. *Blood*. 1992;79:2237-2245.

24. Guthrie TH Jr, Oral A. Immune thrombocytopenia purpura: a pilot study of staphylococcal protein A immunomodulation in refractory patients. *Semin Hematol*. 1989;26:3-9.

25. Brambell FW, Hemmings WA, Morris IG. A theoretical model of gamma-globulin catabolism. *Nature*. 1964;203:1352-1354.

26. Jones EA, Waldmann TA. The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. *J Clin Invest*. 1972;51:2916-2927.

27. Simister NE, Mostov KE. An Fc receptor structurally related to MHC class I antigens. *Nature*. 1989;337:184-187.

28. Israel EJ, Wilsker DF, Hayes KC, Schoenfeld D, Simister NE. Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn. *Immunology*. 1996;89:573-578.

29. Ghetie V, Hubbard JG, Kim JK, Tsen MF, Lee Y, Ward ES. Abnormally short serum half-lives of IgG in beta 2-microglobulin-deficient mice. *Eur J Immunol*. 1996;26:690-696.

30. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc Natl Acad Sci USA*. 1996;93:5512-5516.

31. Hansen RJ, Balthasar JP. Intravenous immunoglobulin mediates an increase in anti-platelet antibody clearance via the FcRn receptor. *Thromb Haemost*. 2002;88:898-899.

32. Hansen RJ, Balthasar JP. Pharmacokinetic/pharmacodynamic modeling of the effects of intravenous immunoglobulin on the disposition of antiplatelet antibodies in a rat model of immune thrombocytopenia. *J Pharm Sci*. 2003;92:1206-1215.

33. Bussel JB. Another interaction of the FcR system with IVIG. *Thromb Haemost*. 2002;88:890-891.

34. Vaughn DE, Bjorkman PJ. High-affinity binding of the neonatal Fc receptor to its IgG ligand requires receptor immobilization. *Biochemistry*. 1997;36:9374-9380.

35. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci*. 2004;93:2645-2668.