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

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Received January 31, 2001; accepted March 22, 2001

This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

The pharmacokinetics, pharmacodynamics, and platelet binding of 7E3, an anti-glycoprotein IIb/IIIa (GPIIb/IIIa) monoclonal antibody, were studied in the rat in an attempt to develop a quantitative animal model of immune thrombocytopenia (ITP). 7E3, a murine IgG1 antibody developed against human GPIIb/IIIa, demonstrated cross-reactivity with rat platelets by flow cytometry and via enzyme-linked immunosorbent assay. The apparent affinity (Kₐ) of 7E3-rat platelet binding was 1.2 ± 0.2 · 10⁻⁷ M⁻¹, with 3.3 ± 0.3 · 10⁴ binding sites per platelet. Following intravenous 7E3 administration (0.8, 4, and 8 mg/kg), plasma concentrations declined in a bi-exponential manner, with a terminal half-life of 61 ± 5 h and a steady-state volume of distribution of 62 ± 15 ml/kg. Clearance was dose-dependent, with values ranging from 0.64 ± 0.08 ml/h/kg (8 mg/kg) to 1.01 ± 0.08 ml/h/kg (0.8 mg/kg). 7E3 induced a reproducible, severe thrombocytopenia in rats and extended bleeding in a manner consistent with human ITP. Nadir platelet counts were 79 ± 33, 25 ± 6, and 17 ± 2 · 10⁶/ml, for 7E3 doses of 0.8, 4, and 8 mg/kg, respectively. Bleeding times after a 10-mm tail incision ranged from 5 ± 3 min in control animals to 15 ± 0 min (the maximum allowed time in this study) in animals receiving 8 mg/kg. Blood volumes lost during bleeding experiments ranged from 30 ± 24 μl (control) to 349 ± 358 μl (8 mg/kg). A reproducible, quantitative rat model of ITP has been created; this model is expected to facilitate the evaluation of new treatments for this disease.

Immune thrombocytopenic purpura (ITP) is a common autoimmune disease, with approximately 33,000 new cases diagnosed each year in the United States (George et al., 1996). Thrombocytopenia in ITP develops as a result of enhanced platelet destruction, precipitated by the interaction of autoantibodies and platelet antigens. The resulting thrombocytopenia is often dramatic and is associated with a variety of hemorrhagic sequelae, including epistaxis, petechiae, gastrointestinal bleeding, and intracranial hemorrhage. The current “standard” therapies for the disease, corticosteroid immunosuppressive therapy and splenectomy, are associated with significant morbidity and are not effective for 25 to 30% of patients with chronic ITP (McMillan, 1997). Intravenous administration of pooled human immunoglobin (IVIG) provides a transient increase in platelet counts for a large fraction of ITP patients; however, the high cost of this therapy prevents routine administration to individuals with chronic ITP. At present, no feasible alternative therapy is available to treat chronic ITP that is refractory to standard therapy, and fatal hemorrhage occurs in approximately 16% of affected patients (McMillan, 1997).

Despite significant need for the development of new therapies for ITP, little progress has been made since the discovery of IVIG activity in 1981. The mechanism(s) of IVIG action are poorly understood; consequently, more specific (and perhaps less costly) therapies derived from nonhuman sources have not been developed. Clinical investigations of new ITP therapies are complicated by several factors. First, a high fraction of ITP patients (e.g., ~30–40% of acute cases) experience spontaneous resolution of ITP symptoms, without therapeutic intervention (George et al., 1996). Second, like other autoimmune diseases, the severity of ITP appears to wax and wane with time, as evidenced by a spontaneous oscillation of patient platelet count. This natural fluctuation in apparent disease severity confounds the quantitative evaluation of new treatments for ITP.

ABBREVIATIONS: ITP, immune thrombocytopenic purpura; GPIIb/IIIa, glycoprotein IIb/IIIa; IVIG, intravenous immunoglobulin; ELISA, enzyme-linked immunosorbent assay; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; Rₐ, total receptor concentration; CL, clearance; AUC, area under the 7E3 plasma concentration time curve; MRT, mean residence time; Vss, volume of distribution at steady state.
vation of new treatments. Third, although it is accepted that patients with very low platelet counts are more likely to experience hemorrhage, no suitable surrogate marker has been definitively associated with patient risk for severe hemorrhage. Finally, no adequate assays exist for quantification of anti-platelet antibodies (Raife et al., 1997). Thus, it is impossible to evaluate treatment effects on autoantibody production or elimination.

Considering the difficulties associated with evaluating ITP treatments in man, it may be advantageous to assess new therapies in animal models of the disease, which may allow systematic, quantitative investigation. Suitable animal models would provide a reproducible condition of severe thrombocytopenia, mediated by anti-platelet antibodies. Ideally, the model should allow accurate and precise quantification of the anti-platelet antibodies, as such a model may facilitate a mechanistic evaluation of therapy. Some animal models of thrombocytopenia have previously been developed, including models that produce thrombocytopenia by nonimmune-mediated mechanisms (Meade et al., 1991; Kuter and Rosenberg, 1995) and by immune-mediated mechanisms (Ashida and Abiko, 1975; Oyaizu et al., 1988; Agam and Livne, 1992; Hosono et al., 1995; Alves-Rosa et al., 2000; Coetzee et al., 2000; Nieswandt et al., 2000). However, to our knowledge, no previous model of ITP is quantitative with respect to both anti-platelet antibody kinetics and the time course of thrombocytopenia.

Our goal was to develop an animal model of thrombocytopenia that is mediated by anti-platelet antibodies and that is quantitative in nature. Such a model may allow for mechanistic evaluation of existing therapies (e.g., IVIG), as well as provide a foundation for the development of new therapies of ITP. In an attempt to develop a quantitative, anti-platelet antibody-mediated animal model of ITP, we have investigated the pharmacokinetics, pharmacodynamics, and platelet binding of a monoclonal anti-platelet antibody in the Sprague-Dawley rat.

Materials and Methods

Production and Purification of 7E3

7E3-producing hybridoma cells were obtained from the American Type Culture Collection (ATCC no. HB-242, Manassas, VA). BALB/c mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were prepared for ascites production by an intraperitoneal injection of Freunds Incomplete Adjuvant (Sigma Chemical, St. Louis, MO), 0.4 ml/mouse, 1 week before injection of antibody-producing cells. Hybridoma cells, 1 × 10⁶ cells/mouse in sterile 0.9% NaCl, were then injected intraperitoneally into the mice. Ascitic fluid was collected 15 days after injection of the hybridoma cells.

Anti-GPIIIa/IIIb IgG was purified from ascitic fluid using protein G chromatography (Pharmacia Biotech Hi-Trap protein G column, Uppsala, Sweden) and a Bio-Rad medium pressure chromatography system. The loading buffer was 20 mM Na₂HPO₄ (Sigma), pH 7.0; the elution buffer was 0.1 M glycine (Bio-Rad), pH 2.8. Purity of the IgG pool was assessed via electrophoresis (SDS-PAGE), using a 12% gel and standard techniques (Harlow and Lane, 1988). Antibody concentration was determined from sample absorbance at 280 nm, with the assumption that 1.35 AU = 1 mg/ml 7E3 (Harlow and Lane, 1988).

ELISA Procedure

Rat plasma 7E3 concentrations were determined using a previously reported ELISA for mouse IgG in rat plasma, with slight modification (Hansen and Balthasar, 1999). Briefly, Nunc Maxisorp 96-well microplates (Nunc model 4-42404, Roskilde, Denmark) were coated with rat anti-mouse antibody (Pierce M-3534, 1:500 in a 20 mM Na₂HPO₄ (PB), 0.25 ml/well) and incubated at 4°C overnight. Samples and standards (0.25 ml/well) were then added, and the plates were incubated for 2 h at room temperature. Goat anti-mouse antibody-alkaline phosphatase conjugate (Sigma A-1682, 1:500 in PB, 0.25 ml/well) was added and allowed to incubate for 1 h at room temperature. p-Nitrophenyl phosphate (Pierce, 4 mg/ml in diethanolamine buffer, pH 9.8, 0.2 ml) was placed in each well, and the change in absorbance with time (over 10 min) was monitored via a microwell plate reader at 405 nm (Spectra Max 250, Molecular Devices, Sunnyvale, CA). Standards were made by diluting a stock 7E3 solution with PBS to the appropriate concentration (0, 10, 20, 40, 60, and 75 ng/ml), and by adding rat plasma (Hilltop Laboratories, Scottsdale, PA) to a final concentration of 1% (v/v). Standard curves were linear in this concentration range. Intra-assay variability was approximately 10% at the limit of quantitation (10 ng/ml).

Specific Activity of 7E3 Preparation

The fraction of antibody specifically directed against platelets (i.e., the specific activity) was assessed by ELISA. Briefly, 7E3, ~1 μg/ml in PBS, was incubated with ~5 × 10⁹/ml outdated human platelets (American Red Cross, Salt Lake City, UT) for 2 h, while shaking at room temperature. Samples were centrifuged at 13,000 g for 6 min to pellet out the platelets and the platelet-bound 7E3. Supernatant (i.e., unbound) 7E3 concentrations were determined by ELISA. Control mouse IgG, obtained from normal mouse blood, was incubated with platelets as a negative control. The specific activity was defined as the percentage of the IgG bound to the platelets.

7E3 Binding to Rat Platelets

Flow Cytometry. Rat platelets were obtained from the blood of female Sprague-Dawley rats (Harlan Sprague-Dawley, Inc.). Blood was obtained either from a jugular vein catheter or via aortal venipuncture into a final concentration of approximately 10 units/ml heparin. Blood was centrifuged at ~650g for 2 min, platelet rich plasma was isolated, and the process was repeated. Platelets were washed with PBS twice, and resuspended to a final concentration of 1% (v/v). Standard curves were linear in this concentration range. Intra-assay variability was approximately 10% at the limit of quantitation (10 ng/ml).
tain from The Scientist's fit of the data. Control mouse IgG was incubated with rat platelets to detect possible nonspecific binding.

**Pharmacokinetic Analysis**

Female Sprague-Dawley rats, 220 to 250 g, were instrumented with jugular vein cannulas under ketamine/xylazine anesthesia (75-15 mg/kg). Two to 3 days following surgery, animals were given an i.v. bolus dose of 7E3 (0.8, 4, or 8 mg/kg). Blood samples (0.3 ml) were collected at 0, 1, 3, 6, 12, 24, 48, 72, 96, and 168 h and placed into 50 μl of an acid-citrate-dextrose anticoagulant. The blood was then centrifuged at 13,000 g for 2 min, and the plasma was isolated and stored at 4°C until analysis (usually within 3 days). Plasma 7E3 concentrations were determined via ELISA, and the resulting 7E3 concentration values were adjusted for anticoagulant dilution by assuming a hematocrit value of 0.46.

Cannula patency was maintained by flushing the cannulas with ~0.15 ml of heparinized saline after each blood draw (10 units/ml during the first 24 h of heavy sampling, and 500 units/ml during daily sampling). In the rare case when cannula patency could not be maintained for the duration of the study, blood (10–20 μl) for pharmacokinetic analysis was taken from the rats’ tails. Following the 0.8-mg/kg dose, 7E3 concentrations at 168 h fell below the limit of quantification of the ELISA, and they were not used in the subsequent pharmacokinetic analyses.

Noncompartmental approaches were used to analyze 7E3 concentration versus time data. Clearance (CL) was determined from the dose of 7E3 and the area under the 7E3 plasma concentration time curve (AUC): CL = Dose/AUC. Terminal half-life values were determined from the relationship \( t_{1/2} = ln(2)/l \), where \( l \) is the negative of the slope of the terminal portion of the ln C versus time plot. The terminal portion of the curve was defined as the final three time points for each rat. Mean residence times (MRT) were estimated from the quotient of AUMC and AUC (Gibaldi and Perrier, 1982), where AUMC is the area under the 7E3 concentration vs time versus time curve. AUC and AUMC values were determined using the linear trapezoidal method (Gibaldi and Perrier, 1982). Volume of distribution at steady state (\( V_{ss} \)) values were determined from the relationship \( V_{ss} = MRT \times CL \). (Gibaldi and Perrier, 1982). Three rats were used for each dose, and the values for the pharmacokinetic parameters are reported as mean ± standard deviation.

**Pharmacodynamic Analysis**

Three pharmacodynamic measures were chosen to evaluate the effects of 7E3 in the rat: platelet count nadir values, bleeding time values following a standardized incision in the tail, and blood volumes lost during the bleeding experiments. Rats (n = 3–5 per group) were dosed with 7E3 as described above. Control rats were given a similar volume of normal saline and were treated in the same manner as the dosed animals.

**Platelet Counts.** Platelet counts were determined over the first 24 h of the study using a Coulter Z1 particle counter (Beckman Coulter, Inc., Fullerton, CA). Briefly, an initial 20-fold dilution of blood was made into Coulter Isoton II solution, and the solution was centrifuged at ~400 g for 5 min. The supernatant was then further diluted with Coulter Isoton II solution, and the resulting sample was analyzed using the Coulter counter (70-μm aperture; counting window of 1.478–2.800 μm). Nadir percent platelet count values were determined from the quotient of the nadir platelet count value and the initial platelet count value for each rat (multiplied by 100).

**Bleeding Times.** Bleeding times were determined using a method similar to that described by Booth et al. (1996). Bleeding times were taken 24 h after the initial dose of 7E3. A 1 cm long × 1 mm deep template-guided incision was made in the tails of anesthetized rats (25 mg/ml ketamine, titrated i.v.), 1 cm from the tip of the tail, ~45 degrees from the dorsal vein. Tails were immersed in 45 ml of normal saline (37°C), and the time until bleeding stopped was measured with a stopwatch. A maximum of 15 min was allowed for bleeding times, as the same animals were being used for the ongoing pharmacokinetic studies. Direct pressure was applied to the cut to stop the bleeding in the animals that bled longer than 15 min.

**Blood Volume Lost.** The volume of blood lost by each rat during the bleeding time measurement was determined spectrophotometrically (Rybak and Renzulli, 1993). Following the bleeding time experiment, 1 ml of 5% Triton X-100, in saline, was added to the container with the blood. After ~30 min of shaking gently, samples were centrifuged at ~6000 g for 5 min and analyzed at 546 nm on a Carey UV-Visible spectrophotometer. Standard curves were generated from the blood of each rat, and blood volume lost was determined from a linear regression analysis of the standards.

**Statistical Analysis**

The pharmacokinetic parameter values and platelet count nadir values obtained in this study were tested for statistical significance at the α = 0.05 level using one-way analyses of variances (Instat, GraphPad Software Inc., San Diego, CA). A Dunnett’s Multiple Comparisons test was used to compare the platelet count nadir values at each dose with the control value.

**Results**

**Production, Purification, and Specific Activity of 7E3**

Approximately 1 mg of pure mouse IgG was obtained from each mouse injected with 7E3-producing hybridoma cells. The purity of each IgG preparation was determined by SDS-PAGE. A representative gel comparing the ascitic fluid, the nonretained protein pool from the protein G purification, and the retained protein pool (IgG) is shown in Fig. 1. For determination of the specific activity, the purified 7E3 preparation was incubated with human platelets. Following the removal of platelet-bound IgG by centrifugation, 7E3 concentrations in the supernatant were determined, and were 4 to 10% of the initial values. In similar experiments, recovery of control mouse IgG was complete. These results, taken together, suggest that the specific activity of the 7E3 preparation was 90 to 96% (90–96 mg of 7E3 per 100 mg of total mouse IgG).

**Binding of 7E3 to Rat Platelets**

Binding of 7E3 to rat platelets was assessed qualitatively using flow cytometry, and resulting histograms of fluores-
cence intensity versus number of particles are shown in Fig. 2. The relative median fluorescence intensity of the 7E3-coated rat platelets was ~33 (Fig. 2A), compared with a value of ~4 for the mouse IgG negative controls (Fig. 2B). The relative median fluorescence intensity of the 7E3-coated human platelets (positive control) was ~760 (Fig. 2C).

The binding curve resulting from the quantitative 7E3-rat platelet binding experiment is shown in Fig. 3. Nonlinear least-squares fitting of the data resulted in an apparent affinity of $1.2 \pm 0.2 \times 10^7 \text{M}^{-1}$ for the 7E3-rat platelet interaction, and a total receptor concentration of $5.3 \pm 0.5 \times 10^{-7} \text{M}$, corresponding to $3.3 \pm 0.3 \times 10^4$ 7E3 binding sites per platelet. No binding of control, polyvalent, mouse IgG to rat platelets was observed.

**Pharmacokinetic Analysis**

Plasma 7E3 concentration verses time data resulting from initial doses of 0.8, 4, and 8 mg/kg are plotted in Fig. 4. Table 1 lists the CL, $V_{ss}$, and $t_{1/2}$ parameter values obtained from noncompartmental analyses of the 7E3 pharmacokinetic data. Dose-normalized plots of the 7E3 concentration verses time data did not superimpose, suggesting dose-dependent pharmacokinetics. Clearance decreased with dose, from a mean value of $1.01 \pm 0.08 \text{ml/h/kg}$ (0.8 mg/kg) to $0.64 \pm 0.08 \text{ml/h/kg}$ (8 mg/kg). This decrease in CL with dose was statistically significant ($p = 0.0043$) and suggests concentration-dependent, saturable clearance of 7E3. Because of this con-
centration-dependence, the reported CL values should be considered as time-averaged values. The methods used to estimate MRT and \( V_{ss} \) are based on assumptions of linear disposition; consequently, the values of MRT and \( V_{ss} \) reported should be regarded as rough approximations (Cheng and Jusko, 1988). The disposition of 7E3 will be more completely characterized in future work that will attempt to build a suitable integrated compartmental pharmacokinetic model and pharmacodynamic model of the 7E3-induced thrombocytopenia. Dose dependencies were not observed for \( V_{ss} \) (62 ± 15 ml/kg) or for \( t_{1/2} \) (61 ± 5 h), with \( p = 0.21 \) and \( p = 0.87 \), respectively.

**Pharmacodynamic Analysis**

**Platelet Counts.** Platelet counts decreased in a dose-dependent fashion upon administration of 7E3, with statistically significant decreases relative to control at each dose (0.8 mg/kg, \( p < 0.05 \); 4 mg/kg, \( p < 0.01 \); 8 mg/kg, \( p < 0.01 \)). Platelet count nadirs occurred 1 to 3 h after administration of 7E3. Nadir values were 54 ± 4, 22 ± 8, and 18 ± 4% of the initial platelet counts for the 0.8-, 4-, and 8-mg/kg doses, respectively (see Fig. 5). In 7E3-treated rats, absolute platelet counts fell to 79 ± 33, 25 ± 6, and 17 ± 2 \( \times \) 10⁶/ml (0.8, 4, and 8 mg/kg, respectively). Therefore, the 4- and 8-mg/kg doses of 7E3 reduced platelet counts to levels associated with severe thrombocytopenia in humans (i.e., < 30 \( \times \) 10⁶/ml). Nadir values for the control group averaged 67 ± 10% of initial platelet counts. Plots of the time course of thrombocytopenia following 7E3 administration are shown in Fig. 6.

**Bleeding Times.** Bleeding times increased in a dose-dependent manner (relative to control) after treatment with 7E3 (Fig. 7). The bleeding times after the 0.8-, 4-, and 8-mg/kg doses of 7E3 were 11.5 ± 4.3, 14.7 ± 0.6, and 15 ± 0 min, respectively. Because of the ongoing nature of the pharmacokinetic studies on these animals, the maximum allowed bleeding time was set at 15 min; this accounts for the decrease in the standard deviation of the bleeding time values with increase in dose of 7E3. Control animals bled for 5.3 ± 2.9 min.

![Fig. 5. Time course of thrombocytopenia produced by administration of saline (○) and 0.8 (○), 4 (□), or 8 (△) mg/kg doses of 7E3 to rats. Rats were given an i.v. dose of 7E3, and platelet counts were obtained using a Coulter Z1 particle counter. The control and treated groups consisted of five and four rats, respectively. Error bars represent the standard deviation of the mean.](image)

![Fig. 6. Nadir percent platelet counts obtained after 7E3 (0.8, 4, or 8 mg/kg) administration. Control and treated groups consisted of five and four rats, respectively. Error bars represent the standard deviation of the mean value. Each dose resulted in statistically significant decreases in platelet counts relative to control.](image)

![Fig. 7. Bleeding times following a 1-mm \( \times \) 1-cm incision in the tail of the rat. Control and treated groups (0.8, 4, and 8 mg/kg 7E3) consisted of three to four rats/group. A maximum of 15 min was allowed for the bleeding time studies. Error bars represent the standard deviation of the mean.](image)

**Blood Volume Lost.** Blood volume lost was assessed from a standard curve relating blood concentration (v/v) to absorbance at 546 nm. Standard curves were created for each individual rat and were linear from 0 to ~1 ml of blood lost (\( r^2 \) values typically >0.999). The blood volume lost verses dose of 7E3 also increased in a dose-dependent fashion (Fig. 8), with values of 87 ± 46, 147 ± 39, and 349 ± 358 \( \mu \)l for the 0.8-, 4-, and 8-mg/kg doses of 7E3, respectively. Saline-treated animals lost an average of 30 ± 24 \( \mu \)l of blood during the bleeding time experiments.

**Discussion**

The main goal of the present work was to develop a quantitative, reproducible, passive immune model of thrombocytopenia in rats that will allow mechanistic evaluation of new therapies for ITP. To accomplish this goal, it was essential to
identify a stable, reproducible, well characterized antibody with reactivity for rat platelet antigens. No anti-rat platelet monoclonal antibody-producing cells are commercially available; consequently, a murine anti-human platelet monoclonal antibody, 7E3, was selected and evaluated for use in developing a rat model of ITP.

7E3 is derived from a commercially available hybridoma cell line, is of the IgG1 isotype, and binds to the platelet membrane GPIIb/IIIa complex, a common target of autoantibodies in patients afflicted with ITP (Fujisawa et al., 1991; Hou et al., 1997). The GPIIb/IIIa complex is present on rat platelets and is known to have a high degree of amino acid sequence homology with human GPIIb/IIIa. Cieutat et al. (1993) report that rat and human GPIIb and GPIIIa share 78 and 92% amino acid sequence homology, respectively. 7E3 was first described in 1985, and it bound human platelet GPIIb/IIIa with an apparent affinity of $2.9 \times 10^8 \text{M}^{-1}$. Chimeric Fab fragments derived from the 7E3 antibody are used clinically to treat patients with ischemic cardiovascular disease (Coller, 1985; Mascelli et al., 1998).

Binding of 7E3 to rat platelets was demonstrated using flow cytometry and ELISA. The estimated number of binding sites for 7E3 on rat platelets is similar to the value reported for the number of binding sites for 7E3 on human platelets [n = 41,300 for human platelets (Wagner et al., 1996) versus 33,000 for rat platelets]. However, the apparent affinity of 7E3 for the rat platelet antigens is approximately 24-fold lower than the reported affinity of 7E3 for human platelets ($K_A = 1.2 \times 10^7 \text{M}^{-1}$ for rat platelets versus $2.9 \times 10^8 \text{M}^{-1}$ for human platelets) (Coller, 1985).

Pharmacokinetic characterization of 7E3 revealed a bi-exponential curve (Fig. 3) and apparent dose dependence in 7E3 clearance. The pharmacokinetic parameters and bi-exponential decline in the concentration versus time curve for 7E3 are similar to other reports for mouse IgG pharmacokinetics in the rat (Bazin-Redureau et al., 1997; Caballero et al., 1998). To our knowledge, no previous reports specifically show dose-dependencies in mouse IgG pharmacokinetics in the rat; however, some groups have reported dose-dependent changes in the pharmacokinetics of murine monoclonal antibodies in humans (Trang, 1992).

As introduced above, ITP patients are prone to develop bleeding complications ranging from epistaxis to fatal intracranial hemorrhage. These bleeding complications may be caused by thrombocytopenia and/or by autoantibody-induced changes in platelet aggregation and adhesion (Balduini et al., 1992). In the evaluation of our animal model, we used two common surrogates of bleeding tendency: bleeding time and blood volume lost (following a standardized tail incision). Bleeding time is prolonged by thrombocytopenia or by inhibition of platelets and is often used as a clinical measure of global bleeding tendency (Lavelle and Maclomhair, 1998). Additionally, some researchers report blood loss to be a quantitative and reproducible method of determining bleeding (Rybakan and Renzulli, 1993).

Although some variability was seen with thrombocytopenia, bleeding time, and blood loss, each clearly showed dose-dependent effects upon administration of 7E3. Part of the observed variability in the bleeding time and blood loss could be caused by the use of heparin to keep the cannulas patent. However, every attempt was made to treat the animals as similarly as possible, and the heparin flushes cannot account for the dose-dependent increases in bleeding times and blood lost upon administration of 7E3. In these studies, no attempts were made to determine the extent to which 7E3-induced increases in bleeding are attributable to 7E3 effects on platelet aggregation as opposed to 7E3 effects on platelet count, and it is probable that both factors contributed to the observed increases in bleeding.

As shown in Fig. 6, saline-treated control animals demonstrated an average nadir platelet count of ~70% of the initial values. This drop in platelet counts in control animals was reproducible and is of unknown origin. Volume changes resulting from saline dosing, heavy sampling (three samples within the first 3 h), and fluid replacement may contribute to the thrombocytopenia observed in these animals. Additionally, before drawing each blood sample for analysis, an initial volume of blood (~0.3 ml) was drawn into a clean syringe to remove heparin from the cannula. After withdrawing the sample, the contents of the first syringe were then re-infused into the rat to minimize blood loss. Given that others have reported that platelet activation may result following blood exposure to biomaterials (including Silastic tubing), it is possible that the contact of the blood with the cannula or syringe could lead to platelet aggregation and/or consumption upon the re-infusion blood (Gemmell et al., 1995). Whatever the cause, the degree of thrombocytopenia observed in the control animals is very small compared with that seen following the highest doses of 7E3.

It is important to point out that this acute, passive model of immune thrombocytopenia does not precisely duplicate the human condition of ITP. Most notably, the thrombocytopenia observed in this animal model is produced by exogenous administration of antibody; in the human condition, the anti-platelet antibody is produced endogenously. Consequently, this model will not facilitate investigation of therapies that involve inhibition of antibody production. Despite the inability of this model to fully replicate the human condition, 7E3-induced immune thrombocytopenia is consistent with the observed clinical manifestations of ITP, in that 7E3 decreases platelet count and increases measures of bleeding.
tendency. Consequently, this will be a useful ITP model in that it will allow quantitative assessment of therapies that interfere with anti-platelet antibody-induced thrombocytopenia and bleeding.

In summary, this work demonstrates that 7E3, an anti-human GPIIb/IIIa monoclonal antibody, binds to rat platelets with an affinity of $1.2 \pm 0.2 \times 10^7$ M$^{-1}$. Additionally, an enzyme-linked immunosorbent assay was developed, which allowed for pharmacokinetic analysis of the disposition of 7E3 in conscious rats. Finally, administration of 7E3 to Sprague-Dawley rats produces a dose-dependent, reproducible thrombocytopenia, with increases in bleeding time and bleeding volume.

A significant advantage of this model of ITP is that it is produced by a readily available, well characterized source of anti-platelet antibody, allowing it to be easily adapted for various applications. Because this model provides a unique opportunity to develop quantitative relationships between anti-platelet antibody concentrations and effects, the model is expected to facilitate systematic, mechanistic evaluations of new therapies of ITP.

References


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