The Reversible Binding of Vinblastine to Platelets: Implications for Therapy

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The ability of platelets to adsorb vinblastine has been used to treat patients with immune thrombocytopenia. It is hypothesized that the drug–platelet complex is coated with antibody, taken up by macrophages which are then destroyed by the drug. We gave 16 courses of vinblastine-platelets to six patients with immune thrombocytopenia. Only one patient responded, and therefore we examined possible reasons for the lack of benefit. Using 3H-vinblastine, the kinetics of vinblastine binding to platelets was studied in vitro. The binding of vinblastine to human and rabbit platelets was identical with maximal binding occurring within 10 min at 600 μg/ml vinblastine. Similarly, the plasma half-life of vinblastine in rabbits was close to that reported for man, and therefore, in vivo binding of vinblastine to platelets in rabbits was considered a suitable model for man. Homologous donor rabbit platelets were labeled with 51Cr alone, 51Cr plus vinblastine, or 3H-vinblastine and infused into recipient rabbits. Vinblastine had no effect on 51Cr survival, but all measurable vinblastine had left the platelets within 2 hr of the infusion. These observations suggest that delivery of the vinblastine to the macrophages depends on the platelets being phagocytized before the drug leaves the platelets. This would be likely to occur only in those patients with severe immune thrombocytopenia. Further investigations into this treatment should be directed at methods to maintain the drug within the platelets.

The MAJORITY of patients with idiopathic thrombocytopenic purpura (ITP) respond to corticosteroids, splenectomy, or immunosuppressive therapy. Patients resistant to these forms of therapy may respond to intravenous vincristine, but these drugs can be associated with adverse reactions. A small number of patients with immune thrombocytopenia are unresponsive to all of these therapeutic approaches. Vinblastine is bound to platelets in vitro, a property that has been utilized therapeutically by a group of investigators who infused platelets incubated with vinblastine into patients with immune thrombocytopenia. The rationale of this approach is based on the hypothesis that the platelet–drug complex is coated with free antiplatelet antibody and is therefore phagocytized by macrophages that are then destroyed by the drug.

We infused vinblastine-loaded platelets (VLP) into six patients with platelet-antibody-positive immune thrombocytopenia and found the majority did not respond. The potential reasons for this lack of response were systematically investigated. The amount of infused drug was determined; a rabbit model to study the binding of the drug to the platelet was developed and the in vitro and in vivo binding kinetics of vinblastine studied.

MATERIALS AND METHODS

Patients

Six patients had idiopathic immune thrombocytopenic purpura (ITP). All patients had thrombocytopenia, a nonpalpable spleen, increased bone marrow megakaryocytes, and no clinical or laboratory evidence of secondary thrombocytopenia. The platelet-associated IgG (PAIgG) level was elevated in all patients. PAIgG was assayed by the antiglobulin consumption assay, the normal value is less than 5 fg IgG/platelet.

Preparation of Vinblastine-Labeled Platelets

Vinblastine-labeled platelets (VLP) were prepared as described. Ten to twelve units of random donor ABO, Rh-compatible platelets were pooled and incubated with 40–50 mg of vinblastine (37°C for 60 min) in the dark. The platelets were then centrifuged (150 × g for 10 mins), most of the supernatant plasma removed, and the platelets were resuspended in 100 ml of vinblastine-free donor plasma. The suspension was then immediately infused into the patients. Since this treatment had been described in detail, and the total amount of infused vinblastine known, it was not considered a human experimental procedure.

In Vitro Binding Studies Using Human and Rabbit Platelets

Tritiated-vinblastine sulphate (3H-vinblastine) (Amersham Laboratories, Burlington, Ill., batch 13—one TRK 507) was used as a trace marker for all experiments (radioactive vinblastine: nonradioactive vinblastine, 1:10). The amount of vinblastine within the platelets was determined by counting the solubilized platelet pellet (NCS tissue solubilizer) in a liquid scintillation spectrophotometer (Beckman Instruments, Fullerton, Calif.). The amount of vinblastine was determined by relating the radioactive counts from the platelet fraction to a

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standard curve performed with each experiment. The standard curve was derived by plotting the scintillation count against varying known concentrations of the radioactive:nonradioactive vinblastine. Platelet counts were performed using a ZB1 Coulter counter (Coulter Electronics, Hialea, Fla.).

The amount of vinblastine infused into the patients was determined by incubating an aliquot of the donor platelets with 3H-vinblastine so that the 3H-vinblastine:platelet ratio was equal to that determined by incubating an aliquot of the donor platelets with 3H-vinblastine (Coulter Electronics, Hialea, Fla.). Known concentrations of the radioactive:nonradioactive vinblastine were added to each platelet aliquot, and the binding of vinblastine to human and rabbit platelets was assessed using varying concentrations and times of incubation of the total vinblastine present determined.

The reversibility of vinblastine binding to human platelets in vitro was assessed by washing with platelet-poor plasma (1000 g x 10 min). The binding of vinblastine to human and rabbit platelets was assessed using varying concentrations and times of incubation of vinblastine with human and rabbit platelets (obtained as described below).

The In Vivo Assessment of the Stability of Vinblastine Binding to Rabbit Platelets

New Zealand white rabbits (weight 2-3 kg) were anesthetized with sodium pentobarbital (30 mg/kg) (MTC Pharmaceuticals, Hamilton, Ontario), administered intravenously. Rabbit platelets were isolated, washed and divided into three aliquots. One aliquot was incubated with vinblastine (non-radioactive), 400 μg/ml/10^6 platelets, and labeled with 10 μCi of sodium chromate-51 (New England Nuclear, Boston, Mass.) (30 min, 22°C). The platelets were washed once (1.2% NaCl). The other two platelet aliquots were handled identically except that one was labeled with 51Cr only, and the other was labeled with 3H-vinblastine only.

The platelets were infused into three groups, each containing three rabbits. Blood samples were taken into sodium citrate (3.8%, 9:1, v:v) at intervals over 96 hr following the infusion. A sample was also taken for whole blood platelet count. The platelet-rich plasma was separated by centrifugation (150g x 5 min), and the platelet pellet then separated from the platelet-poor plasma (1000g x 5 min). The radioactivity was determined in both the platelet-poor plasma and the platelet pellet. The mean platelet survival for each group was determined.

The second infusion was followed by fever, hypotension, and a drop in platelet count from 51,000/μl to 15,000/μl over 12 hr. The platelet count rose following the last treatment with VLP but she required maintenance corticosteroid therapy.

The in vivo assessment of the stability of vinblastine binding to rabbit platelets

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Analysis of Binding of Vinblastine to Platelets In Vivo

The binding of vinblastine to rabbit platelets in vivo was tested for best fit using a 1, 2, 3, or multicompartmental model according to the standard program BMDP P3R. This method of kinetic analysis is described in detail in Appendix A.

RESULTS

Patient Response to Vinblastine-Labeled Platelets

Sixteen courses of vinblastine-labeled platelets were given to six patients with immune thrombocytopenia (ITP). All had elevated levels of platelet-associated
IgG (PAIgG) (Table 1). The normal level of PAIgG is less than 5 fg IgG/platelet. Only one patient responded, and she also received high doses of corticosteroids. In this patient the platelet antibody level fell to near normal levels. The 16 courses of platelet transfusions were complicated by seven episodes of adverse effects including fever, hives, hypotension, and hepatitis. Three patients had a fall in platelet count of 30%-80% within 24 hr of platelet transfusions. A brief description of the patients and their clinical outcome is summarized in Table 1.

**Amount of Vinblastine Infused Into Patients**

The amount of platelet-bound vinblastine given to the patients was determined on five occasions in three different patients. It ranged from 7% to 10% of the initial incubating dose, representing an infused dose of vinblastine of 3.5–5.0 mg.

**Kinetics of Vinblastine Binding In Vitro**

The binding of vinblastine was studied in rabbit and human platelets. The amount of vinblastine bound to platelets reached a plateau when concentrations of between 400 and 800 µg/ml of vinblastine were incubated with $10^6$ platelets (Fig. 1). The relationship between the amount of vinblastine added and the

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**Fig. 1.** The relationship between the amount of vinblastine bound to platelets (ordinate) and the initial concentration of vinblastine (abscissa) for rabbit (O—O) and human (●—●) platelets.

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**Fig. 2.** The relationship between the amount of vinblastine bound to platelets (ordinate) and the duration of incubation of the vinblastine with the platelets (abscissa) for rabbit (O—O) and human (●—●) platelets.
Kinetics of Vinblastine Binding In Vivo

The best fit for both the clearance of vinblastine platelets and plasma-vinblastine was determined to be a two-compartmental system defined by the equation

\[ C = Ae^{-at} + Be^{-bt} \]

where \( C \) equals the concentration of the drug; \( A \) plus \( B \) is the y intercept at time 0; \( a \) is the constant associated with the distribution phase of the drug; \( b \) is the constant associated with the elimination phase; \( t \) is the time.

The survival of the rabbit platelets labeled with \(^{51}\)Cr only was 96 hr, which was not different from those platelets incubated with vinblastine (400 \( \mu \)g/ml/10^6 platelets; Fig. 4). In contrast, all measurable \(^3\)H-vinblastine had eluted from the platelets within 2 hr. The vinblastine binding to the platelets had an alpha phase half-life of 1 min, and a beta phase half-life of 31 min (Fig. 5). The half-life for the plasma vinblastine was longer with an alpha half-life of 4.2 min, a beta half-life of 107 min (Fig. 5).

DISCUSSION

The ability of vinblastine to bind to platelets was utilized by Ahn and associates in an attempt to deliver successive decline in the amount of vinblastine bound to platelets following each of three washes with vinblastine-free platelet-poor plasma. After three washes, 70% of the vinblastine had been removed from the platelets (Fig. 3).
the drug–platelet complex selectively to macrophages. We infused 16 courses of vinblastine-loaded platelets (VLP) to 6 patients and failed to achieve benefit in five. Similar observations have been made by Slichter and associates, who reported that only 2 of 10 patients with immune thrombocytopenia achieved a prolonged remission following VLP, although 6 achieved a transient response. One explanation for the difference between our findings and those reported by Ahn was that our patients did not have immune thrombocytopenia. However, all had elevated levels of platelet associated IgG and three ultimately achieved remission with alternative forms of therapy. Another explanation for the difference is that the vinblastine washed off the platelets during preparation. However, parallel experiments using tritiated vinblastine indicated that 7%–10% of the labeling dose was administered with the infused platelets. This corresponded to a vinblastine dose of 3–5 mg.

To investigate possible reasons for the lack of efficacy of this treatment, the stability of the binding of vinblastine to platelets was investigated in vitro using human platelets, and in vivo using rabbit platelets since it was felt that 1H-vinblastine-loaded platelets could not ethically be administered to our patients. In vitro, the binding of vinblastine to human platelets was readily reversible with 70% removed after 3 washes with platelet-poor plasma. The binding of vinblastine to rabbit platelets in vivo was considered to be a suitable model for man, since the in vitro binding of vinblastine to human or rabbit platelets was similar, reaching a maximum within 10 min at 600 μg/ml vinblastine. Other investigators have reported that the binding of vinblastine to rat platelets is completely reversible and in equilibrium with plasma vinblastine. Therefore, an important consideration in the applicability of the rabbit model to the human situation is the plasma clearance of vinblastine. We determined that the plasma half-life of vinblastine in rabbits was similar to that reported for man (for rabbits and man, respectively, α = 4.2 min; 4.5 min; β = 107 min; 190 min), supporting the suitability of this model. The in vivo experiments in rabbits demonstrated that virtually all vinblastine had left the platelets within 2 hr of infusion. The loss of vinblastine from the platelets was not due to increased clearance of the platelets since they had a normal survival as measured by sodium chromate.

The treatment with vinblastine was associated with a number of adverse effects, including immediate reactions that ranged in severity from urticaria to a severe hypotensive episode. One patient developed hepatitis, which may have been secondary to the multiple platelet units (over 30) that this patient received. While the risk of hepatitis would have been lowered by the use of single donor platelets, it should be emphasized that platelet transfusions are not without potential adverse effects. The febrile and urticarial episodes in several of our patients may have been caused by leukocyte fragments in the platelet preparation, and it is possible that these effects could have been diminished by further centrifugation. The most disturbing adverse effect was the sudden drop in platelet count, which occurred in a number of our patients. The reason for this is uncertain, but it is unlikely that it was caused by the vinblastine since none of the rabbits had a drop in platelet count. We have also observed a sudden worsening of the thrombocytopenia in patients with immune thrombocytopenia who are administered platelet transfusions. We speculate that this may be caused by absorption of immune complexes comprised of antiplatelet antibody and platelet fragments to the surface of the patient’s own platelets.

These observations indicate that the delivery of the drug to the macrophage require the platelet to be phagocytized before the drug leaves the platelet. Previously reported platelet survival studies in patients with immune thrombocytopenia suggest that this would occur only in those patients with the most severe thrombocytopenia.

Future investigation into the management of ITP...
using vinblastine-loaded platelets should be directed at 
techniques to maintain the drug within the platelet. 
Vincristine may prove a more clinically useful agent 
than vinblastine, since it has recently been 
demonstrated that vincristine is maintained within the plate-
let for a longer period of time than vinblastine.23

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APPENDIX A.

ANALYSIS OF BINDING OF VINBLASTINE TO 
PLATELETS IN VIVO

The binding of vinblastine to rabbit platelets in vivo was tested for 
best fit using a 1, 2, 3, multicompartamental model according to the 
standard computer program BMD P3R. This computer program 
tests the "fit" of the clearance data to a variety of multieponential 
equations. Using "feathering" techniques, the curve is divided into 
linear segments that correspond to different "compartments" of 
clearance. The data are analyzed to see if it is best analyzed by a 1, 
2, 3, or multicompartamental model of clearance. This sequential 
analytic process is stopped when a statistically significant improve-
ment in data fitting no longer occurs.14 Since the data are fitted by a 
mathematical "trial and error" iterative process, then good initial 
estimates of the parameters for computer analysis are important.

The initial estimates of clearance that are used as starting points 
for the analysis are obtained by arbitrarily analyzing varying groups 
of data points to conform with varying compartment models. This is 
termed the technique of exhaustive stripping.17

Whether the data can adequately be described by a single, a two, 
or a multicompartamental model is tested statistically by the F test in a 
sequential procedure.15 For example, if a single compartmental 
model provides a good description of the data points, then enlarging 
the system into a two or three compartmental model is redundant 
and makes analysis of clearance unnecessarily complex.

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