

Lack of Efficacy of High-Dose Intravenous Immunoglobulin Treatment of Severe Thrombocytopenia in Patients with Secondary Dengue Virus Infection

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Abstract. Because most cases of secondary dengue virus infection are associated with an increased level of platelet-associated IgG, a high dose of intravenous immunoglobulin (IVIG) may have an effect on the development of severe thrombocytopenia in this disease. A randomized, controlled study was conducted with two treatment groups consisting of a treatment (IVIG) group ($n = 15$) and a non-treatment (non-IVIG) group ($n = 16$) to determine whether a high dose of IVIG is effective in hastening the recovery from thrombocytopenia in patients with secondary dengue virus infection. No significant difference was found in the baseline demographic data between the two groups. No adverse effect of IVIG was observed, but no effect in hastening the recovery of platelet counts was found in patients with secondary dengue infections. The lack of efficacy of IVIG suggests that platelet clearance by macrophages through Fc γ receptors is not a primary mechanism in this disease.

INTRODUCTION

Dengue virus types 1–4 induce a wide spectrum of clinical manifestations, including hemorrhagic manifestations associated with thrombocytopenia and increased vascular permeability. Secondary infections, which are commonly observed in dengue-endemic areas, are more likely to constitute a risk factor for dengue hemorrhagic fever (DHF).¹ Although dengue virus-induced bone marrow suppression decreases platelet synthesis, an immune mechanism of thrombocytopenia resulting in increased platelet destruction appears to be operative in patients with DHF.^{2,3} This disease is now highly endemic in more than 100 tropical countries, and the number of cases has increased dramatically during the past three decades.^{3,4} More than 1,000 deaths occur annually due to DHF, and no specific treatment is currently available.

The high frequency of elevated platelet-associated IgG (PAIgG) in idiopathic thrombocytopenic purpura (ITP) suggests that PAIgG is involved in the mechanisms of thrombocytopenia.^{5,6} Platelets coated with IgG autoantibodies, which form PAIgG, undergo accelerated clearance through Fc γ receptors that are expressed on tissue macrophages. Intravenous immunoglobulin (IVIG) is currently a widely accepted treatment option for ITP. Therapeutic activity of IVIG in the amelioration of ITP appears to involve the mechanism of competitive inhibition of activating Fc γ receptors on phagocytic macrophages in the mononuclear phagocytic system by IVIG-sensitized erythrocytes.⁷ Recent studies demonstrated that the levels of PAIgG levels were inversely correlated with platelet count in patients in the acute phase of secondary dengue virus infections.^{8,9} An increased level of PAIgG was observed in 73.8–80.8% of patients with secondary dengue virus infection. These data indicate that the formation of PAIgG in patients with secondary dengue virus infection may result in thrombocytopenia due either to plate-

let clearance by macrophages or to platelet lysis. This disease can be classified as a dengue virus-induced ITP. It was hypothesized, therefore, that Fc γ receptor blockade by a high dose of IVIG might inhibit the development of severe thrombocytopenia caused by secondary dengue virus infection. A previous case report had suggested this effect in a patient with dengue fever (DF).¹⁰

MATERIALS AND METHODS

Patients and study design. The present randomized, controlled study was conducted to determine the efficacy of a high dose of IVIG in hastening the recovery of platelets or inhibiting the development of severe thrombocytopenia in patients with secondary dengue virus infection. Thirty-six patients clinically suspected of being infected with dengue virus who fulfilled the inclusion criteria were admitted and enrolled in the study on the first day of admission (day 1) to San Lazaro Hospital (Manila, The Philippines) between October and November 2005. Dengue hemorrhagic fever was diagnosed according to World Health Organization (WHO) criteria.¹¹ There were two inclusion criteria for these patients: 1) an acute phase of dengue illness (within 5 days after the onset of illness) verified by the particle agglutination test for dengue IgM,¹² and 2) severe thrombocytopenia (platelet count between 20,000 μ L and 80,000/ μ L) without prominent manifestation of bleeding or shock. The exclusion criterion was a present history of platelet transfusion either before or after admission to the hospital. The study was reviewed and approved by the Bioethics Committees of San Lazaro Hospital and St. Luke's Medical Center. Parents or guardians of all patients provided written informed consent.

Of the patients enrolled, 34 were confirmed to be infected with the dengue virus infected on the basis of a positive result by IgM-capture enzyme-linked immunosorbent assay or reverse transcription–polymerase chain reaction.^{13,14} Three patients were diagnosed as having primary infections, and 31 patients were diagnosed as having secondary infections by a hemagglutination inhibition test.¹⁵ Laboratory tests were conducted at St. Luke's Medical Center (Quezon City, The Phil-

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ippines). Enrolled patients with secondary infections were randomly assigned to the IVIG group and non-IVIG treatment groups by means of sealed envelopes.

Treatment with IVIG. Human immunoglobulin (2.5 g/vial, Gammamune; Bayer Health Care, Brea, CA) at a dose of 0.4 g/kg/day was given intravenously to each patient in the IVIG group intravenously at a constant rate of 0.1g/kg/hour on day 2, and was continued each day until the fourth day after admission (day 4) for three days. The dose and frequency of IVIG in this study was chosen on the basis of previous investigations of IVIG to treat ITP.^{16,17} Standard treatment including intravenous fluids was given to all patients, regardless of group assignment, according to WHO guidelines.¹¹

Evaluation. To determine the effects of a high dose of IVIG, patients in both groups were requested to remain hospitalized until the seventh day after admission (day 7). The primary measure of efficacy was an increase in platelets. Platelet counts in patients of both groups were monitored daily during hospitalization (days 1–7) and on day three after discharge (day 10) at an outpatient clinic. The target sample size could not be estimated for this study because no previous studies had determined the effects of IVIG on the thrombocytopenia during acute dengue virus infection. An interim target sample size of 30 was chosen to ensure that there would be at least a 70% chance for detecting a difference of 40% (100% versus 60%), with a one-sided alpha level of 0.05, in the frequency of platelet counts higher than 80,000/ μ L on the fourth day after admission in patients with dengue illness and a low platelet count between 20,000 and 80,000/ μ L.^{8,9}

Statistical analysis. All the data are expressed as the mean \pm SD. Differences in the demographic and clinical data between the IVIG group and non-IVIG groups were tested using either a chi-square test or a Fisher’s exact test for nominal variables. Differences in laboratory data between the IVIG and non-IVIG groups were analyzed using a Student’s *t*-test for continuous variables. A *P* value less than 0.05 was considered significant.

RESULTS

Demographic data. Thirty-one patients with secondary infection were randomized to either the IVIG group (n = 15) or the non-IVIG group (n = 16) (Table 1). Ten DF cases and

TABLE 1
Comparison of clinical features of patients with secondary dengue virus infection between the IVIG group and the non-IVIG group*

Parameter	Group		P
	IVIG (n = 15)	Non-IVIG (n = 16)	
Mean age, years (SD)	16.3 (3.6)	14.3 (3.4)	0.108
Male %	53.3	43.8	0.594
Mean body weight, kg (SD)	37.8 (10.6)	37.3 (8.7)	0.891
Days after onset (SD)	3.9 (0.6)	3.7 (1.1)	0.462
Laboratory data on day 1			
Mean platelet count, $\times 10^3/\mu$ L (SD)	54.9 (12.7)	48.0 (13.1)	0.147
Mean AST, U/L (SD)	129.1 (130.5)	124.0 (69.5)	0.893
Mean ALT, U/L (SD)	100.6 (105.2)	59.5 (31.5)	0.146
Days of severe thrombocytopenia (SD)	3.1 (1.0)	2.5 (0.8)	0.11

* IVIG = intravenous immunoglobulin; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Period of severe thrombocytopenia indicates the days of platelet count between 20,000 μ L and 80,000/ μ L.

5 DHF cases (1 DHF I and 4 DHF II) were assigned to the IVIG group, and 9 DF cases and 7 DHF cases (6 DHF II and 1 DHF III) were assigned to the non-IVIG group. The increase in the hematocrit (mean \pm SD) was significantly greater in DHF patients than in DF patients for both treatment groups (30.0 \pm 6.3% versus 13.8 \pm 5.3% in the IVIG group and 29.9 \pm 12.9% versus 8.9 \pm 4.4% in the non-IVIG group; *P* < 0.001). No significant differences were found between the two groups with respect to demographic and laboratory data including peripheral platelet count and levels of aspartate aminotransferase and alanine aminotransferase at day 1.

Treatment with IVIG. Because the lowest platelet counts were found on day 2, the recovery phase, but not the phase of development for severe thrombocytopenia, was evaluated in these patients (Figure 1). Although patients who received IVIG were carefully monitored for adverse events, none were observed in patients in this group either during or after treatment. Despite treatment with a high dose of IVIG, no significant change in the platelet counts between day 2 (day of initiation of IVIG treatment) and day 7 was observed in either treatment group. Likewise, no difference was found in the duration of severe thrombocytopenia between the IVIG and non-IVIG groups (Table 1). No significant difference was found in platelet counts in the DF and DHF subgroups between the IVIG and the non-IVIG groups during the same period.

Levels of PAIgG also were examined in patients in both groups on days 2, 5, and 10. The PAIgG levels (mean \pm SD ng/ 10^7 platelets) increased from baseline on day 2 in both the IVIG and non-IVIG groups (24.5 \pm 15 versus 33.8 \pm 28.6), decreased on day 5 (18.5 \pm 8.4 versus 13.7 \pm 5.2), and returned to normal levels (11.0 \pm 7.8 versus 8.7 \pm 3.7) on day 10, which is consistent with our previous findings.⁹ No significant difference was found in levels of PAIgG in patients in the IVIG group and the non-IVIG group.

DISCUSSION

Although the patients were enrolled in an early phase of the illness (less than 4 days after onset), the effect of IVIG on

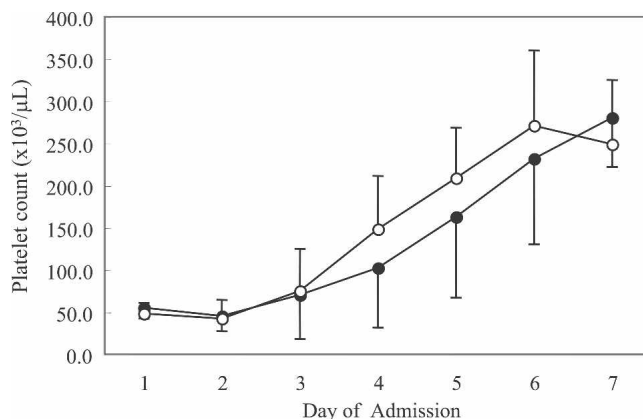


FIGURE 1. Comparison of platelet counts in patients with secondary dengue virus infections receiving a high dose of intravenous immunoglobulin (IVIG) and intravenous fluids (closed circle) and patients receiving intravenous fluids alone (open circle). The IVIG at a dose of 0.4 g/kg/day was given to each patient in the IVIG group on the second, third, and fourth day after hospital admission.

recovery of platelet count could be evaluated, but the inhibitory effect on the development of thrombocytopenia could not be evaluated. No adverse effect of IVIG was noted, but there was no shortening of the time for platelet counts to return to normal levels in patients with secondary dengue infections. Because a rapid recovery of platelet counts is typically found in most patients with severe thrombocytopenia, no additional management of hemostatic abnormalities, including a high dose of IVIG, is required for such patients.

Our group recently developed an *in vitro* assay of phagocytosis of human platelets using flowcytometry. With this assay, it was shown that phagocytosis of platelets from patients with secondary infections by macrophages is significantly increased compared with that of platelets from healthy control subjects (Oishi K. and others, unpublished data). These data suggest that platelet clearance by macrophages plays a role in thrombocytopenia in this disease. Collectively, the lack of efficacy of IVIG in treating severe thrombocytopenia in secondary dengue virus infection shown in this study suggests that platelet clearance by macrophages through Fc γ receptors is not a primary mechanism of thrombocytopenia in secondary dengue virus infection. Other immune mechanisms in this disease may involve platelet clearance by macrophages through complement receptor 3 (CR3) and complement-mediated platelet lysis because complement activation mediated by circulating viral antigen is involved in the pathogenesis of this disease.^{6,18}

Alternatively, de Castro and others recently conducted a pilot study to determine whether anti-D (Rh₀ D) immunoglobulin treatment, which resulted in a platelet increase of more than 70% in Rh+ non-splenectomised patients with ITP, was effective in increasing platelet counts among pediatric and adult patients with dengue illness.¹⁹ Anti-D immunoglobulin also facilitates immune-mediated clearance of antibody-coated erythrocytes and spares sensitized platelets because of preferential destruction of erythrocytes by the mononuclear phagocytic system in ITP.^{7,20} Although de Castro and others demonstrated a trend toward higher platelet counts after treatment with anti-D immunoglobulin among such patients, there was no significant difference in the kinetics of platelet counts between pediatric and adult patients with dengue virus infection who received anti-D immunoglobulin and those who received placebo.¹⁹

In conclusion, the present study demonstrated a lack of efficacy for a high dose of IVIG in hastening the recovery of platelet counts in patients with secondary dengue virus infection. These data suggest that platelet clearance by macrophages through Fc γ receptors is not a primary mechanism of thrombocytopenia in secondary dengue virus infection. Further studies are required to identify the immune mechanisms of thrombocytopenia in secondary dengue virus infection.

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