THROMBOCYTOPENIA ASSOCIATED WITH DENGUE HEMORRHAGIC FEVER RESPONDS TO INTRAVENOUS ADMINISTRATION OF ANTI-D (RH(D)-D) IMMUNE GLOBULIN

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Abstract. Severe thrombocytopenia and increased vascular permeability are two major characteristics of dengue hemorrhagic fever (DHF). An immune mechanism of thrombocytopenia due to increased platelet destruction appears to be operative in patients with DHF (see Saito et al., 2004, Clin Exp Immunol 138: 299–303; Mitrakul, 1979, Am J Trop Med Hyg 26: 975–984; and Boonpucknavg, 1979, Am J Trop Med Hyg 28: 881–884). The interim data of two randomized placebo controlled trials in patients (N = 47) meeting WHO criteria for dengue hemorrhagic fever (DHF) with severe thrombocytopenia (platelets ≤ 50,000/mm³) reveal that the increase in platelet count with anti-D immune globulin (WinRho® SDF), 50 µg/kg (250 IU/kg) intravenously is more brisk than the placebo group. The mean maximum platelet count of the anti-D-treated group at 48 hours was 91,500/mm³ compared with 69,333/mm³ in the placebo group, 75% of the anti-D-treated group demonstrated an increase of platelet counts ≥ 20,000 compared with only 58% in the placebo group. These data suggest that treatment of severe thrombocytopenia accompanying DHF with anti-D may be a useful and safe therapeutic option.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related, but antigenically distinct, virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of the genus Flavivirus. Infection with one of these serotypes does not provide cross-protective immunity, so persons living in a dengue-endemic area can have four different dengue infections during their lifetime. Although dengue fever (DF) is a self-limited febrile illness, dengue hemorrhagic fever is characterized by prominent hemorrhagic manifestations associated with thrombocytopenia and an increased vascular permeability. Secondary infections, commonly observed in the dengue-endemic areas, are more likely to constitute a risk factor for DHF. Although dengue virus-induced bone marrow suppression decreases platelet synthesis, an immune mechanism of thrombocytopenia caused by increased platelet destruction appears to be operative in patients with DHF. An increased level of platelet-associated IgG (PAIgG) is observed frequently in patients with chronic idiopathic thrombocytopenic purpura (ITP) but is also found in a variety of diseases. The major pathophysiologic hallmarks that determine disease severity and distinguish DHF from DF and other viral hemorrhagic fevers are plasma leakage due to increased vascular permeability and abnormal hemostasis. Hypovolemic shock occurs as a consequence of, and subsequent to, critical plasma volume loss. Abnormal hemostasis including increased capillary fragility (positive tourniquet test and easy bruising at the site of venepuncture), thrombocytopenia, impaired platelet function, and consumptive coagulopathy in the most severe form disseminated intravascular coagulation (DIC) contribute to varying degrees of hemorrhagic manifestations. The role of platelets in restoring normal capillary integrity or limiting massive bleeding with DHF has not been adequately described.

Hypothesis. Because anti-D (Rh(D)IgG) immune globulin (WinRho® SDF) is highly effective in producing Fcγ receptor blockade and in raising the platelet count in non-dengue forms of ITP, two placebo-controlled studies in dengue thrombocytopenic patients were conducted to determine if a more rapid platelet recovery ensues after administration of anti-D compared with usual supportive measures only without anti-D.

MATERIALS AND METHODS

Patients and study design. Twenty-seven children (M = 11, F = 16) and twenty adults (M = 10, F = 10) were enrolled after institutional review board approval of the study and informed consent (and assent when applicable) was obtained from all study participants according to the study protocol at De la Salle University Health Sciences Campus (pediatric patients) in Manila and at Davao Medical Center (adult patients), in Davao City, Philippines. Consent was obtained from the caregivers (usually parents) for underage children (< 16 years of age) and, if appropriate, assent from the patient (for underage children). Consent from adults who were not in shock was obtained by a third party not affiliated with the study or, for adults in shock or otherwise not able to provide informed consent, from the next of kin or legal representative. Diagnosis of dengue infection was performed at screening with a Dengue Duo IgM and IgG Rapid Strip Test (PNABIO, Sinnamon Park, Queensland, Australia) and confirmed with an IgM and IgG capture enzyme-linked immunosorbent assay (ELISA) (PNABIO). Thrombocytopenia was confirmed when a manual platelet count was ≤ 100,000/mm³ and severe thrombocytopenia was defined for these studies when the platelet count was ≤ 50,000/mm³.

The diagnosis of DHF was confirmed by the presence of hemorrhagic signs such as petechiae, wet purpura, epistaxis, menorrhagia, or a positive tourniquet test. Dengue shock syn-
Anti-D (Rh(-)–D) is a sterile, freeze-dried gamma globulin (IgG) fraction of human plasma containing antibodies to Rh(-) (D), prepared by Cangee Corporation (Winnipeg, Canada) by an anion-exchange column chromatographic method. The product potency is expressed in international units by comparison to the World Health Organization (WHO) standard. The conversion of “µg” to “IU” is 1 µg = 5 IU. WinRho® SDF is licensed for the treatment of immune thrombocytopenic purpura (ITP) in the Philippines, Canada, the United States, and approximately 20 other jurisdictions worldwide. In the current studies, anti-D was administered at a dose of 250 IU/kg (50 µg/kg) by the Minibag system. To maintain the blind, a translucent Minibag system was used because WinRho® SDF is clear to opalescent in color.

The thrombocytopenia of ITP is believed to be due to accelerated platelet destruction of opsonized platelets with PAIg in the patient’s reticuloendothelial system (RES), chiefly the splenic macrophages. Although the mechanism of action of anti-D is not fully described, it is believed to act by selective blockade of the Fcy receptors in splenic macrophages and other sites of the RES. The anti-D attaches to the red blood cells in Rh-positive (D-positive) patients and preferentially blocks platelet destruction by sacrificing the patient’s RBCs. The expected decrease in the hemoglobin (Hgb) in previous studies conducted in patients with ITP ranged from 11 to 17 g/L. It was anticipated that a similar mechanism of action may decrease the rate of platelet destruction with the thrombocytopenic purpura associated with DHF, as was observed with other forms of secondary ITP.

Placebo consisted of an equal volume of normal saline to the calculated volume of anti-D also administered through a translucent Minibag system.

Both the investigational drug (WinRho® SDF) and the placebo were prepared by a pharmacist who maintained the blind and allocated study drug/placebo with a predetermined randomization code.

Statistical analysis. The safety and efficacy outcomes for each treatment group were compared with the outcomes for each corresponding placebo group.

In the statistical analysis, Fisher’s exact test was used to compare the proportions and the exact binomial distribution was used for calculation of the 95% two-sided confidence intervals for the proportions. The two-sample t-test was used to compare mean values, and normal distribution was assumed to calculate the 95% two-sided confidence intervals for the mean values. All analyses were done in SAS, version 8.2 (SAS, Inc., Cary, NC).

The subgroup analyses were done based on subjects’ baseline platelet count. The data for each study group (adult or children) was stratified by platelet count at entry into the studies such that severe thrombocytopenia (defined as ≤ 50,000 platelet/mm³) or moderate thrombocytopenia (> 50,000 to ≤ 100,000 platelet/mm³). The data for each study was then combined and stratified by platelet count. The data presented in subsequent sections will be limited to children and adults with severe thrombocytopenia (platelets ≤ 50,000 mm³), and the combined data will be presented for both severe (platelets ≤ 50,000 mm³) and moderate (platelets of > 50,000 to ≤ 100,000/mm³) thrombocytopenia.

With a small number of patients in the interim analysis, all the statistical analyses were not intended to be statistically powered. The efficacy and safety data were used to estimate sample size for a larger trial in the future and to identify the most appropriate outcome measures for efficacy and safety. The sample size calculation was done based on the subgroup analysis results using NCSS PASS 2005 (Kaysville, UT).

RESULTS

Safety. Because of the expected extravascular hemolysis, which occurs when anti-D is administered to Rh-positive individuals, the safety of administration of anti-D in this patient population with a hemorrhagic disorder was our first concern. The mean maximum Hgb decrease in the anti-D arms of combined data was 19.6 g/L, whereas in the placebo group it was 17.2 g/L. The findings of larger Hgb decreases in the adult placebo group with severe thrombocytopenia were not statistically significant and were likely due to an outlier in the placebo group who sustained a Hgb decrease of 6.3 g/L prior to death (see Case 2 described in this section).

Two (2 of 47) deaths occurred during these studies: one child and one adult. The overall fatality rate was 4.3%, a rate that is consistent or lower than most tertiary-care centers in dengue-endoemic geographic regions.

1. The first fatality was a 5-year-old female child enrolled into the study with a platelet count of 36,000/mm³. There was epigastric discomfort, hepatomegaly with loose stools on examination and the child was incoherent, restless, and irritable. Activated partial thromboplastin time was 113.7 seconds (ref range 26–37.2 seconds), direct bilirubin and transaminases were elevated (dir bili) at 12.10 µmol/L (ref range 0.0–7.0 µmol/L), AST 1223 U/L (ref range 14.0–59 U/L), ALT 697 U/L (ref range 9.0–72.0 U/L). Serum proteins were low, reflecting increased vascular permeability: total protein 45 g/L (ref range 63–83 g/L), albumin 23 g/L (ref range 35–50 g/L), and globulin 21 g/dL (ref range 23–35 g/dL). The patient was randomized to the anti-D arm with a Hgb of 116 g/L; 24 hours after dosing, Hgb had decreased to 94 g/L with platelet count of 34,000/mm³. Cause of death, which occurred 48 hours after dosing, was attributed by the investigator to complications of DSS and was unlikely to be associated with the administration of anti-D.

2. The second fatality was a 23-year-old female who presented with symptoms of DSS (pulse pressure ≤ 20 mmHg, bp 80/60) and a platelet count of 20,000/mm³. This patient had a petechial rash, bleeding from the gums, vaginal bleeding, and crackles in the right lung base. Hgb was 144 g/L, prothrombin time was elevated to 19.0 seconds (ref range 10–13.4 seconds), as was the APTT at 142.9 seconds (ref range 30.5–46.7 seconds). Transaminases were elevated: AST 116 U/L (ref range 15–37 U/L) and ALT 580 U/L (ref range 30–65 U/L). Severe hypoproteinemia was present at screening visit: total protein 37.7 g/L (ref range 64–82 g/L), albumin 21 g/L (ref range 34–50 g/L), globulin 16.70 g/L (ref range 20–34 g/L); 24 hours after dosing with placebo, the patient experienced renal failure [BUN 8.42 mM/L (ref range 2.5–6.4 mM/L), creatinine 210.7 µmol/L (ref range 53–115 µmol/L)], liver failure [total bilirubin 77.3 (ref range 0.0–17 µmol/L, AST 8 U/L (ref range 15–37), ALT 18 U/L (ref range 30–65 U/L)]. Hemorrhage
continued with Hgb decreasing to 100 g/L and platelet count of 53,000/mm³. Patient died at 48 hours after receiving placebo, with the cause of death attributed to DSS.

Both of these cases illustrate the difficulty in the medical management of advanced cases of DHF and DSS.

Efficacy Endpoints (Table 1)

In these studies, response was defined as an increase in platelet counts by 20,000/mm³ over baseline values after 48 hours of study drug administration.

Children with severe thrombocytopenia (Figure 1). In children with severe thrombocytopenia, the platelet response in the anti-D–treated group is greater at 24 hours and this becomes more pronounced by 48 hours and at subsequent time points, when compared with placebo group. The mean maximum platelet count within 48 hours was 105,200/mm³ in the anti-D arm and 72,800/mm³ in the placebo group; 80% of the cohort responded in the anti-D group, compared with 40% in the placebo group. The mean time to increase platelet counts by 20,000/mm³ from baseline was 36 hours in the anti-D group and 62 hours in placebo group. The mean peak platelet count for the period of observation was 166,400/mm³ in anti-D compared with 138,800/mm³ in placebo. The mean maximum Hgb decrease in the anti-D group was 22 g/L (18.5 g/L in placebo).

Adults with severe thrombocytopenia (Figure 2). The mean maximum platelet count in adults with severe thrombocytopenia within 48 hours of study drug administration was 81,714/mm³ in the anti-D arm and 66,857/mm³ in the placebo group; 71% of the cohort responded in the anti-D and placebo groups. The mean time to increase platelet counts by 20,000/mm³ from baseline was also similar in both groups (43 hours after WinRho® SDF and 40 hours after placebo). The mean peak platelet count for the period of observation was 140,143/mm³ for anti-D compared with 90,857/mm³ in placebo. The mean maximum Hgb decrease in the anti-D group was 15.8 g/L (21.6 g/L in placebo).

Adults and children with severe thrombocytopenia (Figure 3). Pooling of the data from both studies (adult and children) with platelet counts < 50,000/mm³ at entry into the studies again reveals a separation in platelet response between anti-D treatment and placebo by Day 1, and this becomes more pronounced over time. The mean maximum platelet count in adults and children with severe thrombocytopenia within 48 hours of study drug administration was 91,500/mm³ in the anti-D arm and 69,333/mm³ in the placebo group; 75% of the anti-D group responded compared with 58% of the placebo group. The mean time to increase platelet counts by 20,000/mm³ from baseline was 40 hours for anti-D compared with 50 hours for placebo. The mean peak platelet count for the period of observation was 151,083/mm³ for WinRho/H23041 SDF compared with 110,833/mm³ for placebo. The mean maximum Hgb decrease in the anti-D group was 18.9 g/L (20.3 g/L for placebo). This difference in Hgb decrease is not significant and can be attributed to an outlier in the placebo group who sustained a decrease in hemoglobin of 63 g/L.

Adults and children with platelet counts > 50,000 and ≤ 100,000 (Figure 4). The mean maximum platelet count in adults and children in both studies within 48 hours of study drug administration was 147,308/mm³ in the anti-D arm and was 158,400/mm³ in the placebo group; 92% of the anti-D

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<th>Criteria</th>
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<td>Mean maximum platelet count (27,408, 305,216)</td>
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<tr>
<td>Mean time to increased platelet count by 20,000 from baseline (26, 30)</td>
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<td>Mean peak platelet count (27,584, 305,216)</td>
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<td>Mean maximum hemoglobin decrease (g/L)</td>
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group responded compared with 90% of the placebo group. The mean time to increase platelet counts by 20,000/mm$^3$ from baseline was 29 hours for anti-D compared with 32 hours for placebo. The mean peak platelet count for the period of observation was 177,462/mm$^3$ for anti-D compared with 188,700/mm$^3$ in placebo. The mean maximum hemoglobin decrease in the anti-D group was 20.2 g/L (13.6 g/L for placebo).

**DISCUSSION**

The patients randomized in these studies represent secondary dengue infections because both the IgM and IgG capture enzyme-linked immunosorbent assay (ELISA) were positive. The enrolled patients represent ≈50% of the patients admitted to the respective hospitals during the months of the study. Secondary dengue is the population most at risk for developing thrombocytopenia and hemorrhagic symptoms. Typical presentations of secondary dengue infections may include 48–72 hours of dehydration due to anorexia and severe vomiting and hemoconcentration. Patients typically present for admission at the end of the febrile phase with moderate thrombocytopenia despite the hemoconcentration. As the febrile phase ends, hydration will improve and the platelet counts may plummet due to hemodilution and/or the administration of colloid. During the hemodilution phase, patients are at highest risk of developing life-threatening hemorrhages (largely GI). The optimal time to treat the thrombocytopenia
in patients with DHF may be at the end of the febrile stage because it is expected that there will be a further decrease in platelet counts in the ensuing 48 hours. One significant unexplained issue is the difference in response between patients with severe and moderate thrombocytopenia. Although it is possible that patients with moderate thrombocytopenia may have had declining platelet counts at presentation, these patients more likely represent patients with a less severe dengue infection and may not warrant significant treatment to alleviate their thrombocytopenia.

The use of platelet concentrate has been abused by many clinicians despite data from the Philippines and abroad that indicates there is no role for a prophylactic platelet transfusion in DHF. A targeted approach of correcting the thrombocytopenia during the most critical phase of recovery from dengue infections is appealing to clinicians in view of the fact that, at presentation, clinicians cannot predict which patients may progress to severe thrombocytopenia with hemorrhagic symptoms. Anti-D (Rh0-D) is particularly interesting to clinicians because of its relatively low cost when compared with other immune globulin fractions therapies, such as IVIG and the current worldwide shortage of IVIG. The clinical practice of the investigators of this study has changed subsequent to these findings, and we recommend the prescription of WinRho® SDF instead of other blood products, such as platelet transfusion, for patients with severe thrombocytopenia during defervescence. A recalculation of sample size based on the study results was performed to better define the endpoints and to determine sample size requirements for future studies. Based on the proportion of responders in children, group sample sizes of 23 severely thrombocytopenic patients would be needed to achieve more than 80% power to detect a difference of 40%. For the entire population, group sample sizes of 106 would be needed to achieve more than 80% power to detect a difference between the treatment and placebo group proportions of 0.17.
Based on the results and sample size recalculation, the studies were terminated and potential follow-up studies are being evaluated.

CONCLUSION

Dengue hemorrhagic fever is a serious vector-borne infection that may carry a mortality rate from 12% to 44% when accompanied by DSS. Although the hemorrhagic symptoms may only partly be attributed to the severe thrombocytopenia that accompanies DHF, the role of disseminated intravascular coagulation and “leaky capillary syndrome” has not been clearly defined. We have demonstrated a trend toward higher platelet counts after anti-D treatment in children with severe thrombocytopenic purpura when compared with placebo. This trend was not as noticeable in adults with severe thrombocytopenia. When the data for adults and children was pooled, however, there is a trend to higher platelet counts in the anti-D–treated groups. In the aggregate, when hemorrhagic symptoms are life threatening, elevation of the platelet count with therapeutic strategies such as anti-D (Rh0-D) may be one of several strategies available to the clinician treating these very ill patients. The role of anti-D (Rh0-D) in effecting improved outcome of patients with DHF awaits further study.

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Disclosure: Maurice Genereux and Jitendra Dixit are full-time employees of Cangene Corporation, manufacturers of the anti-D immune globulin WinRho® SDF. This statement is made in the interest of full disclosure and not because the authors consider this to be a conflict of interest.

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