Evaluation of the effects of and earliest response rate to anti-D treatment in children with chronic idiopathic thrombocytopenic purpura: a pilot study

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In this pilot study, 30 (14 male, 16 female; median age: 8 years, range: 2-18) chronic non-splenectomized idiopathic thrombocytopenic purpura (ITP) patients with Rh+ blood group and their 49 attacks were evaluated after intravenous (IV) anti-D (WinRho SDF, Cangene Corporation, Winnipeg, MB, Canada) treatment at a dose of 50 μg/kg x 3 days (n=21 cases; 35 attacks) or a single dose of 75 μg/kg (n=9 cases; 14 attacks) to define the hemostatic dose of anti-D. Five of 30 patients (22/49 attacks) were resistant to steroid, intravenous immunoglobulin (IVIG) and vincristine treatment. Hemoglobin (Hb), white blood cells (WBC), platelets (plt) and reticulocytes (ret) were evaluated before and after treatment during the follow-up in sequences on the 1st, 7th, 14th and 21st days after anti-D treatment if the patients had no symptom. All patients, even the resistant ones, experienced an increase in plt count to provide protection from bleeding (≥20x10^9/L in patients with symptoms, ≥10x10^9/L in patients without symptoms). The plt responses of one resistant and five non-resistant patients treated with a single 75 μg/kg dose of IV anti-D in 8 attacks were monitored at the 2nd, 4th, 8th, 24th and 48th hours of the treatment. A protective plt level was attained within 2 hours in 6 attacks of five non-resistant cases and in 24 hours in the remaining 2 attacks of one resistant case. This pilot study suggests that anti-D treatment in ITP patients is effective and can increase plt to a level adequate enough to protect from hemorrhage within 2 hours, when given in a 75 μg/kg dose. A few adverse events (i.e. chills, hemolysis and hemoglobinuria) resolved without intervention.

Key words: chronic idiopathic thrombocytopenic purpura, children, anti-D, earliest response rate, adverse effects.

Idiopathic immune thrombocytopenic purpura (ITP) is a common bleeding disorder in childhood. Cerebral hemorrhage, as the most serious complication of ITP, is minimal (0.5%), but this rate may increase if follow-up and therapeutic intervention are insufficient[1]. Disease that has progressed longer than six months is accepted as chronic ITP. Many therapeutic options may be effective in temporarily raising the platelet (plt) counts; each may at times appear superior or result in serious adverse effects, and thus must be monitored on an individual basis to achieve safe and effective therapy in ITP[2,3]. The questions that have yet to be clarified are as follows: Which agent best accomplishes the goals of treatment and emergent response? How can we better predict outcome and chronicity? While splenectomy can usually induce a long-term remission in most ITP patients, it is

associated with an increased risk of sepsis in children. Although standard or high-dose steroids are considered as the choice of first-line treatment and higher doses are also more effective than intravenous immunoglobulin (IVIG)[4-6], they have side effects, especially when used chronically. IVIG is expensive, its administration is time-consuming, and it has many side effects. Intravenous anti-D immunoglobulin (IV anti-D) has been used under Food and Drug Administration (FDA) license since 1995 in ITP treatment, and it has been preferred to steroid and IVIG in view of its fewer side effects and cost effectiveness and because it can be used on an outpatient basis[7-12]. Reports have pointed at better plt response to anti-D at doses of 75 μg/kg[9,13-15]. In this study, we tried to determine: 1) the rate of plt response to IV anti-D therapy; 2) the time of the earliest hemostatic plt response to anti-D after a single dose of 75 ug/kg; and 3) the occurrence of the serious side effects of anti-D therapy in ITP patients that have come to light, especially in adults[16-19]. We also aimed to evaluate the therapy-related effects in the childhood period.

Material and Methods

In this prospective pilot study, 30 chronic ITP patients and their 49 attacks were evaluated after IV anti-D (WinRho SDF, Cangene Corporation, Winnipeg, MB, Canada) treatment. Non-splenectomized patients with Rh+ blood group were included in the study. Median age was 8 years[2-18] for the 14 males and 16 females. Informed consent was obtained from the parents of all children. Anti-D was administered at doses of 50 μg/kg x 3 days or 75 μg/kg (single dose) in 35 and 14 attacks of 21 and 9 cases, respectively. A single 75 μg/kg dose of IV anti-D treatment was given to the patients if they preferred a short period of treatment. Diagnosis of ITP was made according to the conventional diagnostic criteria, based on patient history and clinical and laboratory examinations[1]. Hematologic parameters were measured using a Beckman Coulter. For evaluation of effectiveness of therapy, two post-therapy plt numbers were used as borderline as: 'hemostatic plt number adequate to prevent hemorrhage', accepted as plt $\geq 20 \times 10^9$/L according to the American Society of Hematology (ASH) Practice Guidelines for patients who had symptoms in attacks[2] and 'Protective plt number for hemorrhage', defined as plt $\geq 10 \times 10^9$/L in a patient who had no symptoms with pretreatment plt level of $< 10 \times 10^9$/L[20]. Each therapy application was accepted as an attack. Children who could not attain these plt numbers after receiving steroids, IVIG and vincristine or those who attained plt count $\geq 20 \times 10^9$/L that decreased to the initial levels within approximately a one-week period were considered as 'resistant to therapy'. Characteristics of the patients are shown in Table I A-C. The group of non-resistant patients consisted of 25 cases (27 attacks). Five patients (22 attacks) were resistant to steroid, IVIG and vincristine treatment. The duration of ITP before administration of the IV anti-D was a median 12 months (7-72 months). All patients had been given steroids or IVIG treatment approximately one month before IV anti-D treatment except for 5 resistant patients for whom therapy was given 1-2 weeks before being included in the study (Table IA). Anti-D doses according to cases and attacks are shown in Table IB. None of the patients had an additional disease that might have given rise to any hemoglobin (Hb) decrease. For all patients, Hb, white blood cell (WBC), plt, and reticulocyte (ret) counts and peripheral smear were examined before therapy and on the 1st, 7th, 14th, and 21st days after treatment. Initial hemotological parameters are given in Table IC. In the non-resistant group (27 attacks), anti–D doses of 50 μg/kg x 3 days were used in 19 cases in 21 of 27 attacks and 75 μg/kg dose was used in 6 cases in 6 of 27 attacks. In the resistant group (22 attacks), anti–D doses of 50 μg/kg x 3 days were used in 2 cases in 14 of 22 attacks and 75 μg/kg dose was used in 3 cases in 8 of 22 attacks. Plt response rates according to dose of anti-D treatment are shown in Table II. As is seen in Table III, 6 patients (8 attacks) receiving anti-D at a dose of 75 μg/kg were monitored closely at the 2nd, 4th, 8th, 24th and 48th hours of the treatment; one of them was resistant to other previous therapies (Table III, Case 4 attack numbers 5 and 6). Before 75 μg/kg of IV anti-D treatment, 6 patients (7 attacks) were given prednisolone (0.5 mg/kg and total dose not exceeding 20 mg) and antipyretics as premedication. Anti-D was given as IV bolus infusion within 3-5 minutes. Kidney functions, blood urea nitrogen and serum creatinine were also examined

Table I. Characteristics of the Patients Treated with Anti-D

(A): Clinical characteristics	Non-Resistant Cases (n=25)	Resistant Cases (n=5)	Total Cases (n=30)
Age (year) median/range	8.5 (2-18)	4 (3-10)	8 (2-18)
Gender (M/F)	11M/14F	3M/2F	14M/16F
Attacks (n)	27	22	49
Duration of ITP (month) before treatment (median/range)	15.5 (7-72)	7 (7-48)	12 (7-72)
(B): Anti-D doses	Non-Resistant Cases	Resistant Cases	Total Cases
50 μg/kg x 3 days	19 cases/21 attacks	2 cases/14 attacks	21 cases/35 attacks
75 μg/kg (single dose)	6 cases/6 attacks	3 cases/8 attacks	9 cases/14 attacks

(C): Initial hematological parameters of anti-D treatment

	Non-Resistant Cases (mean±SD)	Resistant Cases (mean±SD)	Total Cases (mean±SD)
Hb (g/dl)	12.4±1.6 (9.9-16)	12.1±0.97 (9.4-13.9)	12.3±1.38 (9.4-16)
Ret (%)	1.4±1.4 (0.04-4.6)	1.03±0.88 (0.4-2.8)	1.26±1.23 (0.04-4.6)
WBC (mm^3)	7480±1936 (5000-11000)	7041±1751 (4400-10000)	7278±1841 (4400-11000)
Plt (mm^3)	6650±4248 (2000-17000)	6523±3763 (1000-17000)	6593±3996 (1000-17000)

Hb: Hemoglobin. Ret: Reticulocyte. WBC: White blood cells. Plt: Platelet.

before and after one week of treatment. Parents were informed to monitor urine color and any reduction in daily urine excretion number, and to report any abnormality for consideration of the patient's progress.

Statistical Analysis

Statistical comparison was performed using the SPSS statistical program. The results are presented as mean ± standard deviation and/or median (range) for both the resistant and non-resistant cases. The statistical significance was determined using the Mann-Whitney U test for independent samples.

Results

Characteristics of the patients, therapy doses, and initial hematological findings in resistant and non-resistant patients are shown in Table I A-C. The mean plt and Hb levels of the total 49 attacks of 30 cases before administration of IV anti-D were 6593±3996/mm^3 (1000-17000) and 12.3±1.38 g/dl (9.4-16 g/dl), respectively (Table IC). Hb decrease on the first day of administration was observed in 24/49 attacks (in 17/30 patients) ranging between 0.1-2.4 g/dl (mean 0.91±0.68). One week after anti-D administration, decrease in Hb was observed in 34/49 attacks (in 23/30 patients) ranging between 0.1-3.6 g/dl (mean 1.34±0.88). There was no difference in Hb rate of decrease according to anti-D dose at 3 weeks. Initial median ret value of the 30 patients was 0.9%. However, ret response at the first week of anti-D treatment (value >2%) was seen in 9 of 30 patients and 11 of 49 attacks. The post-treatment plt counts according to anti-D dose are shown in Table II.

Non-Resistant Group

In the non-resistant group, plt response on the 1st day was rather better in patients who received anti-D in the 75 μg/kg dose than in those who received the 50 μg/kg dose, although the difference was not significant ($p>0.05$). There were no significant differences in the plt count in the 1st week of treatment; however, patients who received anti-D as 50 μg/kg x 3 days had significantly higher plt levels in the following 2nd and 3rd weeks of treatment than

Table II. Platelet Response Rates According to Dose of Anti-D Treatment

Cases	Dose	Platelet values (mm^3) (mean±SD)				
	Case / Attack	Initial	1 day	1 week	2 weeks	3 weeks
Non-resistant (n=25)	50μ/kg x3day (19/21)	7090±4238 (2000-17000)	32428±26 (5000-90000)	149442±161501 (4000-526000)	177000±164164 (17000-498000)	129307±129785 (21000-450000)
	75 μ/kg (6/6)	4800±4207 (2000-12000)	60400±53078 (4000-145000)	79400±85007 (12000-197000)	46600±39462 (15000-94000)	21500±8582 (15000-34000)
Resistant (n=5)	50 μ/kg x3day (2/14)	7214±4154 (2000-17000)	34285±52367 (3000-204000)	96153±115083 (3000-397000)	47181±49241 (3000-152000)	33000±36276 (3000-102000)
	75 μ/kg (3/8)	5142±2544 (1000-8000)	18500±22792 (2000-63000)	27857±32570 (5000-100000)	20142±29054 (8000-86000)	25250±36563 (5000-80000)

those who received single-dose anti-D as 75 μg/kg ($p<0.05$). The attained plt levels with 75 μg/kg single dose anti-D treatment were adequate for hemostasis, but lower than those attained with 50 μg/kg x 3 days. The follow-up of plt response rate for three weeks seemed to depend on dosage. On the other hand, there were no significant differences according to dosage at the following 1st, 2nd, and 3rd weeks in the resistant group ($p>0.05$).

Resistant Group

Patients (n=5) who were resistant to several types of therapy also attained a plt level sufficient enough to protect from hemorrhage for 1-2 months. Nevertheless, one athletically active patient was given anti-D as 50 μg/kg x 3 days, although he had a sufficient plt level for hemostasis (17000/mm^3) and could continue his activities without any hemorrhage after his plt level increased to normal for a satisfactory period. The most important point is that in 8 attacks (75 μg/kg single dose IV anti-D) that were closely monitored, patients attained a protective and/or hemostatic plt level at a very early stage, such as within 2 hours in 6 of 8 attacks and in 24 hours in the remaining 2 attacks of a resistant case (Table III).

Side Effects

The most common side effects, vomiting and fever, were not observed in any patient; chill was observed in 3 patients but it subsided within one hour. Hemoglobinuria was present in 2 patients receiving anti-D as 50 μg/kg x 3 days dose and in 2 patients receiving the treatment as 75 μg/kg single dose. Anti-D was administered at the same dosage to those four patients for the next attacks under premedication. This time neither hemoglobinuria nor severe hemolysis was observed. Kidney functions of patients with hemoglobinuria were at normal levels during their follow-up.

Discussion

In this pilot study, we evaluated response rate, earliest response, response of resistant cases and also the adverse effects of IV anti-D treatment in different doses. According to the results of this study, we can say that all patients, even the resistant ones (n=5), experienced increase in platelet count to a sufficient level to provide protection from bleeding (Table II). On the first post-treatment day of anti-D treatment, comparison of platelet response according to dose in the non-resistant

Table III. Close Monitoring of Platelet (Plt) Response to a Single Dose of Anti-D

Case No	Attacks(n)	Plt (mm^3)					
		0	+2 hour	+4 hour	+8 hour	+24 hour	+48 hour
1	1	8,000	10,000	10,000	15,000	30,000	30,000
	2	8,000	20,000	40,000	40,000	150,000	135,000
2	3	3,000	20,000	30,000	22,000	50,000	48,000
3	4	20,000	23,000	60,000	44,000	145,000	256,000
4*	5	3,000	3,000	10,000	9,000	20,000	20,000
	6	2,000	8,000	12,000	10,000	17,000	22,000
5	7	12,000	30,000	12,000	15,000	20,000	20,000
6	8	3,000	26,000	30,000	27,500	30,000	45,000

*Resistant case

group demonstrated that platelet increase in the group of patients treated with 75 μg/kg was more than that in the group of patients treated with 50 μg/kg, but the difference was not significant ($p>0.05$). This can be explained by the fewer number of attacks in the group of patients treated with 75 μg/kg (6 attacks versus 21 attacks in the group administered 50 μg/kg). However, this result was similar to the studies reported before[9,13-15].

In patients who received anti-D in a single dose of 75 μg/kg, the earliest hemostatic and protective level ($\geq 20x10^9$/L, $\geq 10x10^9$/L in patients with and without symptoms, respectively) from hemorrhage was attained at the 2nd hour in 6 of 8 attacks (Table III). While response rate was as rapid as 2 hours, responses of one resistant case were delayed, but on both occasions he displayed long-lasting drug effect with increased platelet counts, which were nearly equal to that observed in non-resistant cases (Table III). Moser et al.[14] reported that after treatment with 75 μg/kg anti-D in a group of 25 patients with a plt$\leq 15x10^9$/L, 19 of them (76%) responded to treatment, and an increase in platelet count ($>20x10^9$/L) was observed within 6-10 hours.

Decreased hemoglobin level was seen in more than half of the attacks (34/49), but it was not severe, and no patient required blood transfusion. Hemoglobinuria was also detected in 4 of 30 patients in 4 attacks and none of them experienced hemoglobinuria in their next treatment with anti-D.

The most important side effect in patients with anti-D, disseminated intravascular coagulation (DIC), was reported by Gaines[19] 5 of the 6 patients died, with all 5 patients being adults with an underlying disease. One of the 6 patients was a child who survived with appropriate approaches. However, in our study, we did not experience DIC. Chill was detected in 3 patients. All adverse events resolved without intervention. Although hemolysis and platelet increase rate have been reported as likely to be dose-related in a few adults [7,21], in our study, hemolysis was found to be independent from anti-D dose. Interestingly, the hemolytic process was not as severe in the next attacks of the same patients who received the same doses of anti-D, as postulated before [19]. The reason for no similar dose-dependent effect as observed in hemolysis has been explained previously by Freiberg[9]. Response rate in platelet count was dose-dependent in non-resistant cases, as reported by Tarantino[13].

Basic approaches of ITP treatment depend on Fc receptor (R) blockade that allows an acute, dramatic increase in platelet count[21-23]. Clinically important effects of FcR blockade are mainly seen following IVIG and IV anti-D, as well as steroid treatment. The mechanism of acute platelet increase following both IVIG and IV anti-D Ig is thought to be slowing of the destruction of opsonized platelets via effects on the FcγR system. Anti-D and IVIG achieve acute platelet increases by different mechanisms of FcγR interaction[21]. The rationale behind treatment of a patient with IV anti-D is based on its lower cost, less time requirement for administration, and less side effects compared to steroids, and on the presence of a greater proportion of aggregated IgG than IVIG in specific anti-D preparations. Factors affecting hemolysis and platelet response are FcγR polymorphism, Rh phenotype, antibody concentration, binding affinity, number of D-antigen binding sites on the erythrocytes, splenic saturation resulting in diminished capacity for clearance of anti-D–sensitized red blood cells (RBCs), concentrations of IgG1, IgG3, and/or anti-idiotype antibodies in anti-D, which may form complement-fixing immune complexes *in vivo*, and low-titer blood group antibodies, other than anti-D antibodies [17,21-24].

Results of IV anti-D treatment in the childhood period remain preliminary. We evaluated our results as a contribution to the accumulating literature. Our findings were similar with the studies reported before[7-15]. This study pointed out that: 1) all patients were responsive, 2) the earliest platelet response to a level of protection from hemorrhage was attained at the 2nd hour of treatment with 75 μg/kg in non-resistant cases, 3) platelet level also increased to provide hemostasis in resistant cases, 4) adverse events due to therapy subsided without intervention, 5) adverse events and response to anti-D in an individual do not predict or exclude the same response in another attack, but premedication may be effective for side effects; rapidity and duration of response in ITP are clinically as important as the absolute or relative increase in the platelet count, 6) the rapid platelet increase,

such as within 2 hours as was observed with a single 75 μg/kg dose of IV anti-D, seems to indicate that it would be worthwhile in the treatment of children with ITP in emergency, even in resistant cases, and 7) precaution and close follow-up are very important after anti-D treatment according to previous reports that showed severe side effects, though less so in childhood.

REFERENCES

1. Yetgin S, Olcay L, Ozsoylu S, Hicsonmez G, Gurgey A, Tuncer AM. Retrospective analysis of 78 children with chronic idiopathic thrombocytopenic purpura: follow-up from 1976 to 1996. Pediatr Hematol Oncol 1997; 14: 399-412.
2. George JN, Woolf SH, Raskop GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996; 88: 3-40.
3. Blanchette VS, Chir B, Carcao M. Childhood acute immune thrombocytopenic purpura 20 years later. Semin Thromb Hemost 2003; 29: 605-617.
4. Ozsoylu S, Saylı TR, Ozturk G. Oral mega dose methylprednisolone versus intravenous immunoglobulin for acute childhood thrombocytopenic purpura. Pediatr Hematol Oncol 1993; 10: 317-320.
5. Ozsoylu S, Irken G, Karabent A. High-dose methylprednisolone for acute thrombocytopenic purpura. Eur J Hematol 1989; 42: 431-435.
6. Ozsoylu S. High dose intravenous methylprednisolone for treatment of childhood chronic thrombocytopenic purpura. Pediatr Rev Commun 1987; 2: 29-40.
7. Bussel JB, Graziano JN, Kimberly RP, Pahwa S, Aledort LM. Intravenous anti-D treatment of immune thrombocytopenic purpura: analysis of efficacy, toxicity, and mechanism of effect. Blood 1991; 77: 1884-1893.
8. Scaradavou A, Woo B, Woloski BM, et al. Intravenous anti-D treatment of immune thrombocytopenic purpura: experience in 272 patients. Blood 1997; 89: 2689-2700.
9. Freiberg A, Mauger D. Efficacy, safety and dose response of intravenous anti-D immune globulin (WinRho SDF) for the treatment of idiopathic thrombocytopenic purpura in children. Semin Hematol 1998; 35: 23-27.
10. Tarantino MD, Madden RM, Fennewald DL, Patel CC, Bertolone SJ. Treatment of childhood acute immune thrombocytopenic purpura with anti-D immune globulin or pooled immune globulin. J Pediatr 1999; 134: 21-26.
11. El Alfy MS, Mokhtar GM, El-Laboudy MA, Khalifa AS. Randomized trial of anti-D immunoglobulin versus low-dose intravenous immunoglobulin in the treatment of childhood chronic idiopathic thrombocytopenic purpura. Acta Haematol 2006; 115: 46-52.
12. Simpson KN, Coughlin CM, Eron J, Bussel JB. Idiopathic thrombocytopenia purpura: treatment patterns and an analysis of cost associated with intravenous immunoglobulin and anti-D therapy. Semin Hematol 1998; 35: 58-64.
13. Tarantino MD, Young G, Bertolone SJ, et al. A single dose of anti-D immune globulin at 75 μg/kg is as effective as IVIG at rapidly raising the platelet count in newly diagnosed immune thrombocytopenic purpura (ITP) in children. For the acute ITP study group. J Pediatr 2006; 148: 489-494.
14. Moser AM, Shalev H, Kapelushnik J. Anti-D exerts a very early response in childhood acute idiopathic thrombocytopenic purpura. Pediatr Hematol Oncol 2002; 19: 407-411.
15. Newman GC, Novoa MV, Fodero EM, Lesser ML, Woloski BM, Bussel JB. A dose of 75 microg/kg/d of IV-anti-D increases the platelet count more rapidly and for a longer period of time than 50 microg/kg/d in adults with immune thrombocytopenic purpura. Br J Haematol 2001; 112: 1076-1078.
16. Hong F, Ruiz R, Price H, Griffiths A, Malinoski F, Woloski M. Safety profile Win Rho anti-D. Semin Hematol 1998; 35 (Suppl): 9-13.
17. Gaines AR. Acute onset hemoglobinemia and/or hemoglobinuria and sequelae following Rh(o) (D) immune globulin intravenous administration in immune thrombocytopenic purpura patients. Blood 2000; 95: 2523-2529.
18. Kees-Folts D, Abt AB, Domen RE, Freiberg AS. Renal failure after anti-D globulin treatment of idiopathic thrombocytopenic purpura. Pediatr Nephrol 2002; 17: 91-96.
19. Gaines AR. Disseminated intravascular coagulation associated with acute hemoglobinemia or hemoglobinuria following Rh(0)(D) immune globulin intravenous administration for immune thrombocytopenic purpura. Blood 2005; 106: 1532-1537.
20. Bolton-Maggs PH, Moon I. Assessment of UK practice for management of acute childhood idiopathic thrombocytopenic purpura against published guidelines. Lancet 1997; 350: 620-623.
21. Cooper N, Heddle NM, Haas M, et al. Intravenous (IV) anti-D and IV immunoglobulin achieve acute platelet increases by different mechanisms. Modulation of cytokine and platelet responses to IV anti-D by FcγRIIa and FcγRIIIa polymorphisms. Br J Haematol 2004; 124: 511-518.
22. Bussel JB. Fc receptor blockade and immune thrombocytopenic purpura. Semin Hematol 2000; 37: 261-266.
23. Ware RE, Zimmerman SA. Anti-D: mechanisms of action. Semin Hematol 1998; 35: 14-22.
24. Schwartz J, Spitalnik S, Grima KM. Severe hemolysis following administration of Rh_o(D) immune globulin in an ITP patient associated with anti-C. Blood 2006; 107: 2585.