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RESEARCH ARTICLE

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Abnormal platelet aggregation in pediatric pulmonary hypertension

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Abstract

Endogenous prostacyclin stimulates pulmonary vasodilation and inhibits platelet aggregation. For the synthetic analog treprostinil, used in the treatment of pulmonary hypertension (PH), conflicting, anecdotal evidence exists regarding its effects on clinically relevant platelet function. This study investigated whether treprostinil therapy results in inhibition of platelet aggregation in pediatric PH patients. This is a single institution, prospective, cohort study. Pediatric patients ≤18 years of age on medical therapy for PH underwent platelet function testing by light transmission aggregometry with U-46619—a stable analog of endoperoxide prostaglandin H_2, exhibiting properties similar to thromboxane A2 (TXA2). Results were compared for those on continuous treprostinil therapy (TRE) versus those on other, non-prostacyclin therapies (non-TRE). Thirty-five patients were enrolled: 18 in the TRE group and 17 in the non-TRE group. There was no difference in platelet aggregation abnormalities between the two groups: 44% ($n = 8$) in the TRE group and 41% ($n = 7$) in the non-TRE group were abnormal. Furthermore, subgroup analysis showed no difference based on treprostinil dosing. This study demonstrated similar, moderately high rates of abnormal platelet aggregation in pediatric PH patients on continuous treprostinil therapy compared to those on other, non-prostacyclin therapies. The high rate of abnormal platelet aggregation in the entire cohort, however, warrants follow-up study to identify a potential inherent risk in this population.

KEYWORDS
pediatrics, prostacyclin, pulmonary arterial hypertension

INTRODUCTION

The endogenous molecule prostaglandin I_2 (or prostacyclin) is a prostaglandin produced by endothelial cells via prostacyclin synthase from the arachidonic acid derivative prostaglandin H_2. In endothelial cells, prostacyclin activates prostacyclin receptors to increase production of cAMP, which produces smooth muscle relaxation and vasodilation. This provides a beneficial effect in pulmonary arterial hypertension (PAH), where a

reduction in endogenous vasodilators contributes to increased pulmonary vascular resistance.

Prostacyclin also acts on platelets via G protein-coupled receptor activation and signaling of adenylyl cyclase to produce cAMP, which inhibits platelet activation. The actions of prostacyclin are antagonistic to another endogenous molecule, thromboxane A_2 (TXA_2) which activates its receptor and sequentially induces platelet aggregation connected by fibrinogen through activated GP IIa/IIIb receptors in the cell membrane of platelets. Given pathologic remodeling leading to thrombosis of small vessels in end-stage PAH, there is interest in the regulation of platelet function in particular in concert with drug treatment.[1]

Epoprostenol was the first synthetic prostacyclin approved by the Food and Drug Administration for the treatment of PAH. Treprostinil, also an approved synthetic analog of prostacyclin with a longer half-life and multiple routes of administration, has gained favor in the pediatric population. While idiopathic PAH and epoprostenol have been associated with thrombocytopenia,[2,3] the direct effect of current prostacyclin therapies on platelet aggregation has not been evaluated in patients with pulmonary hypertension (PH). Despite the mechanism of action and therefore potential for platelet dysfunction, there is only conflicting, anecdotal evidence as to whether treprostinil causes platelet dysfunction, and if so, whether it is clinically relevant. This study investigated whether, in pediatric PH patients, treprostinil therapy inhibits platelet aggregation when compared with those receiving non-prostacyclin PH therapy.

METHODS

This is a single institution, prospective study conducted between 2015 and 2019. Pediatric PH patients ≤18 years of age and on medical therapy were classified into two groups: those on intravenous or subcutaneous treprostinil (TRE group) and those receiving non-treprostinil/non-prostacyclin therapies (non-TRE group). Patients on any antiplatelet agent (e.g., aspirin) were excluded. IRB approval was obtained; consent and assent were obtained when applicable.

Patient blood samples were collected in citrate tubes and transported at room temperature to the special coagulation lab for testing. Each sample was separated into platelet-rich plasma (PRP) and platelet-poor plasma (PPP). To prepare PRP, whole blood tubes were centrifuged at 170 g for 10 min at room temperature without application of the brake. After removal of the PRP, autologous PPP was then prepared by centrifugation at 1500g for 10 min at room temperature. PRP for testing is adjusted to 250,000 platelets/μl using autologous PPP.

FIGURE 1 PAP-8 light transmission aggregometer (Bio/Data Corporation): This system measures the rate and extent of platelet aggregation, agglutination, and activation stimulated by individual agonists; platelet agglutination/aggregation is indicated by degree of light transmission.

Platelet aggregation was performed on a PAP-8 light transmission aggregometer (PAP-8, Bio/Data Corporation) using 25 μl of agonist added to 225 μl of PRP to induce platelet aggregation (Figure 1). The PRP is exposed to the platelet agonist and as platelets are activated, they aggregate and fall to the bottom of the tube. Light transmission through the sample increases with increased platelet aggregation and can reach up to 100% if light transmission equals that of the patient's PRP. Testing was required to be completed within 4 h of sample collection per lab protocol, and a normal control was analyzed with each run.

The agonist U-46619 is a stable analog of endoperoxide prostaglandin H_2 (with properties similar to TXA_2) and is capable of causing platelet aggregation. U-46619 was chosen for this study to test the ability of treprostinil to inhibit platelet aggregation, as TXA_2 platelet aggregation is blocked in vivo by endogenous prostacyclin. Per Clinical and Laboratory Standards Institute recommendations, two agonist concentrations (1.5 and 2.0 μM) were tested for each patient. Institutional parameters noted normal aggregation to be >65% for TXA_2 1.5 μM and >70% for TXA_2 2.0 μM; that is, TXA_2 1.5 μM has a lower threshold and thus more sensitive detection of abnormal platelet aggregation when compared to the 2.0 μM concentration. Therefore, we disregarded the less sensitive 2.0 μM results and focused on the 1.5 μM

results for the purposes of this study. In addition, disaggregation is reported in patients with abnormal aggregation to evaluate whether the release of TXA_2 from platelets occurred to boost a secondary wave of platelet aggregation.

Patient demographics, classification of PH, recent laboratory data (included if within 2 weeks of platelet function testing), recent hemodynamics (if applicable), and concomitant medications were recorded. Median (range) is reported for non-normally distributed numeric data. Statistical analysis of baseline patient characteristics and platelet function test results were compared between groups using the *t*-test and Wilcoxon rank-sum test for numeric variables, and chi-square test for categorical variables.

RESULTS

Demographics

Thirty-five PH patients were enrolled in the study, 18 in the TRE group (on subcutaneous (17) or IV (1) treprostinil ± other therapies) and 17 in the non-TRE group (non-prostacyclin therapies alone). The median duration of time on treprostinil before platelet function testing is 1.2 years (range 1 day to 5.8 years). Table 1 shows demographics for the cohort, including hemodynamic data from the most recent cardiac catheterization before study enrollment. Thirteen patients in the TRE group and 14 in the non-TRE group were enrolled at the time of catheterization. TRE group consisted of a higher percentage of patients with Group 1 PAH and higher baseline pulmonary vascular resistance and ratio of pulmonary to systemic vascular resistance. Non-treprostinil therapies were similar between groups. There were four patients with Group 5 disease: two (one in non-TRE group) with segmental PH (pulmonary atresia with ventricular septal defect and major aorto-pulmonary collateral arteries) and two with single ventricle congenital heart disease.

Aggregometry results

Out of the 35 total patients, 15 demonstrated abnormal platelet aggregation to TXA_2 1.5 μM, and there was no difference between groups: 8 of 18 (44%) in the TRE group and 7 of 17 (41%) in the non-TRE group ($p = 0.845$). Disaggregation was tested in 12 of 15 abnormal patients, with disaggregation demonstrated in all 12. No difference or relationship was seen in aggregation results based on the dose of subcutaneous/intravenous treprostinil (Figure 2). In

TABLE 1 Demographics

	Group 1 ($n = 18$)	Group 2 ($n = 17$)	*p* Value
Age	9.2 years (1.5–18.5)	5.1 years (1.7–17.6)	0.656
Weight	24.4 kg (9.6–80.2)	17 kg (9.8–63.9)	0.235
Sex	10 (56%) F	8 (47%) F	0.615
WSPH group			
1	15 (83%)	11 (65%)	0.029
2	0	0	
3	0	5	
4	0	0	
5	3	1	
Medications	16	11	0.121
PDE-5	7	5	0.555
ERA	2	0	0.486
Milrinone O_2	6	3	0.443
Hemodynamic data	8 mmHg (3–16)	7 mmHg (2–16)	0.487
RA pressure	36 mmHg (16–115)	30 mmHg (19–58)	0.292
mPA pressure	10 mmHg (5–30)	10.5 mmHg (5–20)	0.576
PCW pressure	7.5 WU (2–29)	4.8 WU (2–7.5)	0.022
PVRi Rp:Rs	0.5 (0.1–2.2)	0.3 (0.1–0.5)	0.013

Note: Values are median (range) or number (percentile) if indicated.
Abbreviations: mPA, mean pulmonary artery; PCW, pulmonary capillary wedge; PVRi, indexed pulmonary vascular resistance; RA, right atrial; Rp:Rs, ratio of pulmonary to systemic vascular resistance.

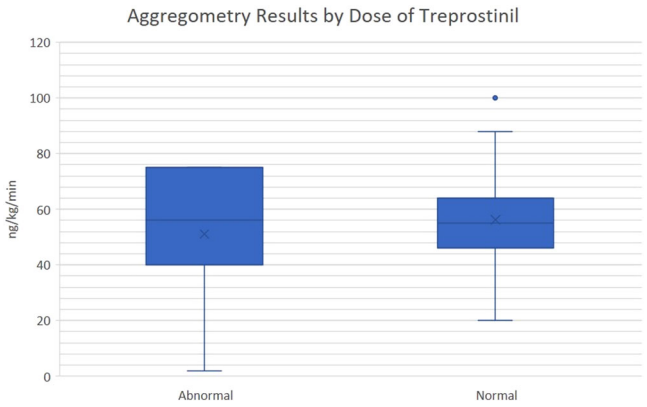

FIGURE 2 Eighteen patients on treprostinil infusion: 8 with abnormal and 10 with normal aggregation results. Dose (*Y*-axis) shown by group (abnormal vs. normal aggregation).

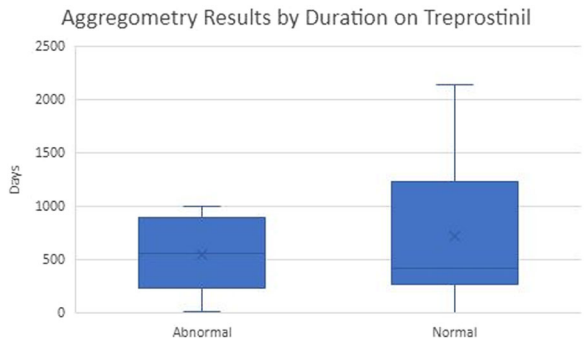

FIGURE 3 Eighteen patients on treprostinil infusion: 8 with abnormal and 10 with normal aggregation results. Duration of therapy (*Y*-axis) shown by group (abnormal vs. normal aggregation).

addition, no difference or relationship was seen in aggregation results based on the duration of treprostinil therapy (Figure 3).

One patient in the TRE group also underwent testing before initiation of treprostinil. Aggregation results were normal under both conditions for this patient.

Laboratory results

At the time of aggregometry, 24 patients had a platelet count and additional coagulation studies. The median platelet count in the TRE group was 161 (range 131–244) and in the non-TRE group was 210 (range 95–307). The median (range) PT, PTT, and fibrinogen in the TRE group was 13.8 (13.6–15.5), 34.7 (30.2–43.6), and 280 (210–610), and in the non-TRE group was 14.9 (13.6–16.4), 33.9 (28.6–41.6), and 245 (188–323), respectively.

DISCUSSION

This study assessed the relationship of platelet function to treprostinil therapy using LTA, the gold standard for assessment of platelet aggregation by measuring light transmission when platelets aggregate following agonist stimulation.[4] There was no difference in the rate of abnormal aggregation between pediatric PH patients on treprostinil therapy versus those on non-prostacyclin therapies. Interestingly, the rate of abnormal platelet aggregation was just over 40% in both groups, suggesting an underlying predisposition to platelet dysfunction, potentially related to their underlying disease though the rate of dysfunction in patients with PH not on PH therapies is unknown.

First described in 1976, the endogenous compound prostacyclin was recognized to be a potent inhibitor of platelet aggregation.[5] Released by vascular endothelium, prostacyclin plays a role in vasodilation and may also maintain vascular integrity by preventing thrombosis. In patients with PH, there is an imbalance in the production of prostacyclin and TXA_2 that may also predispose to thrombosis with higher circulating levels of TXA_2 that promotes platelet aggregation.[6] Additional complicating factors in the hemostatic balance in these patients is that there may be an inherent platelet storage pool deficiency or granule release defect related to PH, independent of drug treatment. Herve et al, in a case report, presented a patient with PH and an inherited platelet storage deficiency associated with a high level of 5-hydroxytryptamine (5-HT) in plasma. Administration of ketanserin, a 5-HT antagonist, substantially reduced the PH.[7]

Previous studies also demonstrate the effect of epoprostenol on reducing platelet activation in extracorporeal life support to reduce circuit thrombosis[8–10] but do not address whether epoprostenol produces clinically significant effects on platelet aggregation in vivo when used as a pulmonary vasodilator. Recently, a study of 26 pediatric patients showed decrease in platelet microvesicles in patients treated with subcutaneous treprostinil, suggesting a beneficial effect of decreased platelet aggregation.[11] This could be beneficial to reduce the risk of intravascular thrombosis seen in end-stage PAH though no prior studies have examined the influence of treprostinil on in vivo platelet aggregation.

While this study reports a seemingly high rate of platelet dysfunction, the clinical significance of abnormal platelet aggregation results is unknown. In addition, the etiology of platelet dysfunction in this population was not explored. It is possible that decreased platelet aggregation occurs in a number of PH patients due to endothelial dysfunction and chronic platelet activation, leading to subsequent platelet degranulation and ultimate decreased function. Vrigkou et al.[12] reported a cohort of PH patients who had significantly decreased aggregation compared to normal controls; this was particularly true for newly diagnosed PH patients.

There are several limitations in this current study, including the relatively small sample size and no true control group to differentiate between PH disease-specific changes versus PH medication effects on platelet aggregation. In addition, although prostacyclin was studied due to its direct inhibition of platelet aggregation through cAMP, the nitric oxide pathway and the phosphodiesterase inhibitors (sildenafil, tadalafil, or milrinone as concomitant medications in this study) may also inhibit platelet function in vivo through

production of cGMP, activation of G-protein coupled receptor, and inhibition of TXA_2.[13–17] Additional evaluation of bleeding risk with a platelet count, coagulation panel was not available for all patients.

Ultimately, the clinical risk of bleeding will be important to further delineate in these PH patients who may have other systemic illnesses or be on other medications that increase the risk for bleeding. In addition, comprehensive prospective monitoring for clinical bleeding would enhance the ability to provide clinical correlation to aggregometry results.

CONCLUSIONS

In summary, in 35 pediatric patients with PH on medical therapy, 43% demonstrated abnormal platelet aggregation, and there was no difference between those on treprostinil therapy compared to those on solely non-prostacyclin therapy. The high rate of abnormal platelet aggregation in the entire cohort warrants follow-up study to identify a potential inherent risk, and to understand the relationship between platelet function and coagulation profile in pediatric PH patients.

AUTHOR CONTRIBUTIONS

All authors made a substantial contribution to the study design, acquisition of data, and analysis of results. All authors critically reviewed the manuscript and accepted this version for submission.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

Parental consent and assent, when applicable, were obtained from all participants. All authors agree upon consent to publication.

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