ELSEVIER

Archives of Medical Research 32 (2001) 268–272

Archives of Medical Research

ORIGINAL ARTICLE

Platelet Function Analysis in Children with Schönlein-Henoch Syndrome

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Received for publication June 2, 2000; accepted February 19, 2001 (00/089).

Background. Schönlein-Henoch syndrome (SHS) or anaphylactic purpura in childhood is the result of pathologic and immunologic responses to different antigens. These antigens could induce the formation of immune complexes responsible for vasculitis and their precipitation on the endothelium of small blood vessels. Purpuric bruises, hematuria, hematemesis, melena, or hematohesis may suggest coagulation disturbances. Increasing bleeding tendency may suggest platelet function disturbance. To examine the qualitative function of platelets in children with SHS, we decided to analyze its aggregation function.

Methods. Using the Born method of testing, we analyzed platelet aggregation in 24 children with SHS.

Results. Based on the aggregograms examined, we observed that most patients had abnormal aggregation curves, in which platelets demonstrated a block of release of the endogenous ADP, with or without desaggregation.

Conclusions. One clinical symptom of SHS appearing in most patients is a mild or increased tendency toward bleeding. On measuring induced aggregation of platelets in children with SHS, we observed that the qualitative function of platelets was disturbed.

Key Words: Schönlein-Henoch syndrome, Thrombocytopathia, Childhood.

Introduction

Although the course of Schönlein-Henoch syndrome (SHS) is unknown, allergy, infection, or drug sensitivity may play an important role in the development of this disease in the majority of patients. In the early 1990s, the American College of Rheumatology (ACR) developed classification criteria for seven forms of vasculitis as well as criteria for classification of Henoch-Schönlein purpura (1–3). ACR criteria for the classification of Henoch-Schönlein purpura (HSP) include the following: age at disease onset <20 years; palpable purpura; acute abdominal pain; gastrointestinal bleeding, and biopsy showing granulocytes in the walls of small arterioles or venules. The presence of any two or more of these criteria distinguishes HSP from other forms of vasculitis (3). It is important to point out that SHS differs from other vasculitic syndromes because of palpable purpura, such as the typical rash on the buttocks and lower extremities. These lesions last from 3 to 30 days. It is predominantly a disease of children and onset frequently occurs between the ages of 2 and 10 years. Prognosis is very good for recovery within weeks or months. The primary manifestation of SHS is small-blood-vessel vasculitis, particularly in those of skin, gastrointestinal tract, and kidney. Vasculitis is the result of the pathologic immunologic response on different antigens including various infectious organisms or drugs; this response triggers developing immune complexes (4–7). Further, these antigens can induce a precipitation of the immune complexes responsible for vasculitis on the endothelium of the small blood vessels in skin, joints, kidneys, ileum, and central nervous system (CNS) (8,9). Casonato demonstrated the presence of abnormally large vWf multimers in platelets and endothelial cells. He pointed out that damage and/or perturbation of endothelial cells was associated with SHS (10).

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0188-4409/01 $–see front matter.
PII S0188-4409(01)00282-X

described in Ishikawa cells were also found expressed in the intact pregnant rabbit on day 5. As TCF levels begin to decline after day 5, perhaps TRBP1 or TRBP2 could be involved in TCF expression regulation, but whether and how these transcription factors (TRBP1, TRBP2, TCF, and TPF/YY1) interact to regulate UG expression remain to be clarified.

Our results show that the transcription factors found in Ishikawa cells are also expressed in the endometrium of the pregnant rabbit and that apparently UG gene expression was controlled throughout pregnancy by two constitutive (TPF/YY1 and Sp3/SpR-2) and two regulated (TCF and Sp1) factors. We solely analyzed factors that bind to the TATA box and GT1 sites (region VI) localized within the UG promoter; however, other factors such as TRBP1 and TRBP2 or perhaps other yet unknown factors may be involved in regulation of this gene *in vivo*.

Acknowledgments

This work was supported by the National Institute of Perinatology, Mexico City, Reg. #212250/07061. The authors wish to thank Drs. Jörg Klug and Guntram Suske for providing the oligonucleotides used in our experiments, as well as Susana González for her help in keeping and caring for the rabbits.

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Spectrum of clinical expression is variable, depending on the degree of reaction, and may vary from a minimal petechial rash to severe gastrointestinal, renal, neurologic, pulmonary, and joint disease (11). Approximately two-thirds of patients develop migratory polyarthritis primarily of the ankles and knees. Skin symptoms are dominant with symmetric purpura most frequently involving the buttocks and lower extremities. Kaku pointed out that 33.5% of patients developed renal involvement from 3 days to 17 months after onset of the disease (12).

Clinical purpuric bruises, hematuria, hematemesis or melena, and hematochezia may suggest coagulation disturbance (13). Some authors (14) have found platelet-associated Ig (PAIg) in 75% of children with SHS and indicate its close relationship with autoimmune disease. However, hemostasis abnormalities observed in SHS are important for understanding the pathophysiology of the disease; these abnormalities include consumption and decrease of plasma coagulation factor XIII (F XIII) (15,16). It was found that the decrease in F XIII level less than 60% of normal was correlated with the high risk of complications (17). Abdominal manifestation with colicky abdominal pain is most common in children with SHS. Bloody diarrhea with intussusception may also occur.

Other authors (18) noted that children with acute-stage SHS had low T-lymphocyte percentages and impaired function, increased B lymphocyte percentage, high immunoglobulin level, and normal or elevated complement level. SHS may vary in the degree of expression of infiltrates in skin or at other localizations as well as in degree of bleeding tendency. To examine the qualitative function of platelets in children with SHS, we decided to analyze its aggregation function.

Materials and Methods

The study was carried out at the Department of Hematology, Oncology, and Immunology at the Pediatrics Clinic of the University Hospital Split, Croatia. Twenty-four children with SHS and normal platelet count were tested for platelet aggregation. Age of patients was 4–12 years and all had palpable purpura. According to ACR 1990, all patients expressed at least two criteria for SHS. Blood samples were taken upon admission and no patient took antiaggregation drugs or a drug that can disturb platelet functionality.

Standard Born method on the Chrono-log machine, model 330 platelet aggregometer (Chrono-Log Corp., Havertown, PA, USA) was performed (19,20). This test measures platelet response to various aggregating agents and is at present the most useful test for platelet function available. Platelet aggregation was measured by detecting transmission of light through stirred platelet-rich-plasma to which an aggregating agent was previously added. This method is semiquantitative and requires a healthy population with normal platelet function testing and the obtaining of the normal values in each laboratory. Referent values in our laboratory for platelet aggregation are as follows: stimulated by ADP 2 μmol/mL for the first phase of aggregation 10–20%, and for the second phase 15–30%, and stimulated by adrenaline 10 μmol/mL for the first phase of aggregation is 10–15% and for the second phase, 30–60%. Slight or no response to standard requires increased concentration of aggregating agent. Patient samples included the following: 1) platelet-rich plasma (PRP), and 2) platelet-poor plasma (PPP).

To prepare platelet-rich plasma, 4.5 mL of patient sample was mixed with 0.5 mL 0.129 M sodium citrate in Becton Dickinson Vacutainer Systems (Franklin Lakes, NJ, USA). PRP was prepared by centrifuging at room temperature at 800 rpm for 10 min. At least 2 mL of plasma is removed and kept in a plastic tube. Aggregation testing must be performed within 3 h. PRP is adjusted to a count of approximately 200×10^9/L with PPP.

On the other hand, to prepare platelet-poor plasma, after separating PRP the remainder of the sample was centrifuged at 3,000 rpm for 10 min. After centrifuging, plasma was removed and kept in a plastic tube until the beginning of analysis. PPP is used for modification of absorption and diluting PRP.

Reagents. To stimulate platelet aggregation adherence of one platelet to another, aggregating agents such as adrenaline and adenosine diphosphate (ADP) were added to PRP that was continuously stirred. Two concentrations of each aggregating agent were used, ADP (2- and 5-mol/mL) and adrenaline (10-, 25-, and 50-μmol/mL). Using the standard concentration of aggregating agent, aggregation proceeds more slowly than with the higher concentration. In case of no response, a higher concentration was used.

CHRONO-PAR platelet aggregation reagents kits (Chrono-Log Corporation) with carefully pre-measured reagents and diluents were used in our experiments. The CHRONO-PAR kit contains color-coded vials with ADP (lyophilized) plus pre-measured diluents and adrenaline plus pre-measured diluents. PPP (0.5 mL) was pipetted into one aggregation cuvette, while 0.5 mL of PRP was pipetted in the other cuvette with a different concentration of aggregating agents (ADP, adrenaline) with the addition of magnetic stick. We placed the cuvette with PPP onto the heating block on the aggregometer. The 100% baselines were set with the PPP, and 0% baselines were set with PRP.

After baseline was observed, an aggregating agent was added (ADP, adrenaline). Upon the addition of 0.05 mL of the reconstituted reagent, the final reaction mixture was carried out as follows: ADP-2 μmol/mL or 5 μmol/mL, adrenaline-10 μmol/mL, or 25 μmol/mL. The platelet-rich plasma was slightly turbid due to the presence of platelets in suspension. When aggregating agent was added, turbidity decreased because of the clumping action of the platelets. PRP was stirred in a cuvette and maintained at a constant temperature of 37°C.

Light increases as it passes through, and this increase in transmitted light was measured and recorded on a linear strip chart recorder. The resultant curve was a record of the rate and amount of aggregation. This procedure was carried out to detect abnormalities in platelet aggregation. Platelets have surface-binding sites for ADP, a natural biologically active platelet aggregating substance, and adrenaline. The process of aggregation was measured spectrophotometrically and the rate and recording device plotted the degree of aggregation. Platelet aggregation varies with different agents and with concentrations.

Platelet aggregation curves were analyzed by dividing the curve into the first and second aggregation phases. Primary or first-phase aggregation refers to the direct aggregation of platelets by ADP or adrenaline. Second phase aggregation is mediated through the release of ADP from the platelets themselves.

Results

Twenty-four children (11 boys and 13 girls) with SHS were treated and tested at the Department of Hematology, Oncology, and Immunology of the University Hospital Split Pediatrics Clinic during the period from 1985 to 1998. All patients enrolled in this study expressed at least two criteria for SHS. Furthermore, all had palpable purpura with petechiae and were <20 years of age. Eleven (45.8%) had joint swelling; ankle, knee, and elbow were mostly involved. Two children (8.3%) had joint pain simultaneously. Three children manifested abdominal pain without blood in the stool. Platelet function was analyzed with standard platelet aggregation tests.

First-phase aggregation induced with ADP was normal in 23 children (95.8%) and slight in one child (4.2%). Second-phase aggregation stimulated by ADP was slight in three patients (12.5%), 14 (58.3%) had a block of endogenous ADP release, and seven had desaggregation (29.2%). Only two patients (8.3%) had normal aggregation curve in second-phase aggregation stimulated with standard concentration of ADP.

Additionally, when we induced platelet aggregation via another stimulus such as adrenaline, we found in first-phase normal aggregation in 19 children (79.2%), slight aggregation in four (16.7%), while in one child aggregation was absent (4.2%). In the second phase of aggregation, we observed normal reactivity in 18 patients (75.0%), slight reactivity in two (8.3%), three patients demonstrated complete block of endogenous ADP release (12.5%), while in one child aggregation was absent (4.2%).

One child of our cohort failed to release endogenous ADP in both first- and second-phase aggregations stimulated with adrenaline and had a block of endogenous ADP release with desaggregation in the second phase stimulated with exogenous ADP. All results are summarized in Table 1.

Discussion

The pathogenesis of most forms of SHS such as vasculitis is still unclear (21). SHS is a very complex disease and there is no single method to be selected in establishing the diagnosis or follow-up studies of either chronic or recurrent forms.

One clinical symptom of SHS in most patients is mild or increased bleeding tendency. In these patients, platelet morphology is normal, while the number of platelets can sometimes be decreased.

Platelet function may be disturbed in a wide variety of disorders such as kidney and liver, and in myeloproliferative and immunologic diseases. Furthermore, the action of various drugs such as aspirin, indomethacin, penicillin, phenylbutasone, and dipyridamole may lead to platelet function abnormalities. Most acquired disorders of platelet function result from abnormalities of platelet granule and membrane proteins. In addition, they are difficult to review and usually appear to be substantially more complex. These disturbances may arise from exposure to drugs or autoantibodies. It is well known that platelet antibodies may affect platelet function. Additionally, platelets and endothelial cells share a number of antigens. Platelets in patients with immune thrombocytopenic purpura (ITP) in remission may have abnormal function, such as aggregation and adhesion defects and IgG from the patient's serum could inhibit the aggregation of normal platelets (22,23). Platelet and vascular disorders demonstrate purpura but no evidence has yet been advanced for involvement of the platelets in immune-mediated endothelium disorders. Weiss in 1980 studied α- and dense granule content in two patients with systemic lupus erythematosus and one with ITP. Platelet α- and dense granule content were reduced in all patients (24).

In our experience based on the examined aggregograms of the 24 children with SHS, we observed that the majority had a block of release of endogenous ADP on ADP as the induction agent, and demonstrated a block of the release of endogenous ADP with or without desaggregation. Only three children manifested a block of release of endogenous ADP on adrenaline as the induction agent.

However, the most consistent defect in our patients with SHS is impaired release of endogenous ADP, suggesting an acquired storage pool defect. Furthermore, platelet dysfunction in patients with purpura anaphylactoides may suggest that an immune-mediated endothelium disorder can attack the platelet, resulting in acquired ADP-release disease. As the results of all factors mentioned previously, it appears that purpura anaphylactoides may lead to fewer active platelets than normal and that immune platelet damage may lead to acquired platelet function defect.

By determining induced platelet aggregation in patients with SHS, we found disturbances in platelet function. Abnormalities present in α- and dense granules may be areas of focus for further research using modern platelet experimental techniques to clarify the nature of granule defects.

Table 1. Clinical features with platelet first- and second-phase aggregation following induction with ADP and adrenaline in children with SHS

Patient	Symptoms of SHS	Platelet first-phase aggregation	Platelet second-phase aggregation
1	Palpable purpura on lower extremities, abdominal pain, knee swelling	ADP 2 μmol/mL 27%; ADP 5 μmol/mL 45%; adrenaline 25 μmol/mL 13%	ADP 2 μmol/mL 2% with desaggregation; ADP 5 μmol/mL 28%; adrenaline 25 μmol/mL 67%
2	Palpable purpura on buttocks, gluteus, and lower extremities, swelling of both ankles	ADP 2 μmol/mL 35%; ADP 5 μmol/mL 52%; adrenaline 50 μmol/mL 18%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 6% with desaggregation; adrenaline 50 μmol/mL 75%
3	Palpable purpura on elbows, lower extremities, abdominal pain	ADP 2 μmol/mL 38%; ADP 5 μmol/mL 45%; adrenaline 50 μmol/mL 25%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 12% with desaggregation; adrenaline 50 μmol/mL 80%
4	Palpable purpura on trunk and lower extremities	ADP 2 μmol/mL 37%; ADP 5 μmol/mL 46%; adrenaline 25 μmol/mL 10%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 34%; adrenaline 25 μmol/mL block of endogenous ADP release
5	Palpable purpura on elbows, lower extremities, both knees, elbows, and right-ankle swelling	ADP 5 μmol/mL 19%; adrenaline 50 μmol/mL 6%	ADP 5 μmol/mL block of endogenous ADP release; adrenaline 50 μmol/mL 5%
6	Palpable purpura on buttocks, lower extremities, swelling of both ankles	ADP 2 μmol/mL 30%; ADP 5 μmol/mL 45%; adrenaline 50 μmol/mL 12%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mlL10%; adrenaline 50 μmol/mL block of endogenous ADP release
7	Palpable purpura over the lower extremities, right-ankle swelling	ADP 2 μmol/mL 14%; ADP 5 μmol/mL 18%; adrenaline 50 μmol/mL 12%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 25%; adrenaline 50 μmol/mL 56%
8	Palpable purpura, ecchymoses on the buttocks, lower and upper extremities	ADP 2 μmol/mL 32%; adrenaline 25 μmol/mL 15%	ADP 2 μmol/mL 37%; adrenaline 25 μmol/mL 69%
9	Palpable purpura on lower extremities	ADP 2 μmol/mL 35%; adrenaline 10 μmol/mL 29%	ADP 2 μmol/mL 62%; adrenaline 10 μmol/mL 66%
10	Palpable purpura on the lower extremities, ankles, elbows, swelling of knees, and pain	ADP 2 μmol/mL 27%; ADP 5 μmol/mL 52%; adrenaline 25 μmol/mL 12%	ADP 2 μmol/mL 22%; ADP 5 μmol/mL 25%; adrenaline 25 μmol/mL 65%
11	Palpable purpura on the buttocks, lower extremities, right-ankle swelling	ADP 5 μmol/mL 50%; adrenaline 10 μmol/mL 13%; adrenaline 25 μmol/mL 13%	ADP 5 μmol/mL 24%; adrenaline 10 μmol/mL block of endogenous ADP release; adrenaline 25 μmol/mL 8%
12	Palpable purpura on lower and upper extremities	ADP 5 μmol/mL 50%; adrenaline 10 μmol/mL 35%	ADP 5 μmol/mL block of endogenous ADP release with desaggregation; adrenaline 10 μmol/mL 85%
13	Palpable purpura on lower and upper extremities and buttocks	ADP 2 μmol/mL 25%; adrenaline 10 μmol/mL absent	ADP 2 μmol/mL block of endogenous ADP release with desaggregation; adrenaline 10 μmol/mL absent
14	Palpable purpura on lower extremities and buttocks, abdominal pain	ADP 2 μmol/mL 32%; adrenaline 10 μmol/mL 18%	ADP 2 μmol/mL 30%; adrenaline 10 μmol/mL 58%
15	Palpable purpura on lower and upper extremities and trunk	ADP 2 μmol/mL 23%; ADP 5 μmol/mL 31%; adrenaline 50 μmol/mL 8%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 55%; adrenaline 50 μmol/mL 92%
16	Palpable purpura on lower extremities, elbows and buttocks, right elbow and ankle swelling	ADP 2 μmol/mL 20%; ADP 5 μmol/mL 56%; adrenaline 50 μmol/mL 58%	ADP 2 μmol/mL 5% with desaggregation; ADP 5 μmol/mL 15%; adrenaline 50 μmol/mL 56%
17	Palpable purpura on lower extremities, elbows and buttocks, and knee swelling	ADP 2 μmol/mL 46%; adrenaline 10 μmol/mL 13%	ADP 2 μmol/mL 29%; adrenaline 10 μmol/mL 50%
18	Palpable purpura on lower extremities, and swelling of both ankles	ADP 2 μmol/mL 45%; adrenaline 50 μmol/mL 25%	ADP 2 μmol/mL block of endogenous ADP release; adrenaline 50 μmol/mL 7%
19	Classic lesions on buttocks, lower extremities, right ankle, and swelling of both feet	ADP 2 μmol/mL 19%; ADP 5 μmol/mL 34%; adrenaline 50 μmol/mL 7%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 26%; adrenaline 50 μmol/mL 48%
20	Palpable purpura on lower extremities and both ankles	ADP 2 μmol/mL 31%; ADP 5 μmol/mL 56%; adrenaline 10 μmol/mL 15%	ADP 2 μmol/mL 35% with desaggregation; ADP 5 μmol/mL 16%; adrenaline 10 μmol/mL 75%
21	Palpable purpura on lower extremities with petechiae	ADP 2 mol/mL 32%; ADP 5 μmol/mL 45%; adrenaline 25 μmol/mL 13%; adrenaline 50 μmol/mL 15%	ADP 2 μmol/mL 4% with desaggregation ADP 5 μmol/mL 12% with light desaggregation; adrenaline 25 μmol/mL 36%; adrenaline 50 μmol/mL 48%
22	Palpable purpura on buttocks, lower extremities, right-ankle and foot swelling	ADP 2 μmol/mL 23%; ADP 5 μmol/mL 65%; adrenaline 25 μmol/mL 15%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 31%; adrenaline 25 μmol/mL 75%
23	Palpable purpura on buttocks, extremities, pain in both ankles and feet	ADP 2 μmol/mL 4%; ADP 5 μmol/mL 8%; adrenaline 50 μmol/mL 5%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL block of endogenous ADP release; adrenaline 50 μmol/mL 4%
24	Palpable purpura with purpuric bruises on trunk, extremities	ADP 2 μmol/mL 27%; ADP 5 μmol/mL 50%; adrenaline 50 μmol/mL 21%	ADP 2 μmol/mL block of endogenous ADP release; ADP 5 μmol/mL 25%; adrenaline 50 μmol/mL 50%

Acknowledgments

We would like to thank P. Martinic at the Cro-Lab in Split, Croatia for contributing data to this study.

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