Erythrocytic Hydrogen Sulfide Production Is Increased in Children with Vasovagal Syncope

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Objectives To explore the differences in erythrocyte hydrogen sulfide (H_2S) production in children with vasovagal syncope (VVS).

Study design A total of 54 children including 27 with VVS, aged 6-16 years (mean age 11.3 ± 3.3 years), and 27 healthy children, aged 3-17 years (mean age 10.4 ± 1.8 years) were included in the study. Children with VVS had symptoms of dizziness, pallor, blurred vision, nausea, and some had syncope. Erythrocyte H_2S production was measured by a sulphur-sensitive electrode. Flow-mediated dilation (FMD) of brachial artery was measured for each patient by vascular ultrasound.

Results H_2S production from erythrocytes was significantly increased in the children with VVS compared with controls ($P < .01$). The erythrocytic H_2S production in the VVS-vasoinhibitory subgroup was obviously higher than that in VVS-cardioinhibitory ($P < .05$) and VVS-mixed inhibitory subgroups ($P < .05$). FMD in the VVS-vasoinhibitory subgroup was greater than that in the VVS-cardioinhibitory ($P < .05$) and the VVS-mixed subgroups ($P < .05$). The erythrocytic H_2S production had a positive linear correlation with FMD in children with VVS ($P < .05$).

Conclusions Increased erythrocyte H_2S production may be involved in the pathogenesis of VVS in children. *(J Pediatr 2015;166:965-9)*.

Vasovagal syncope (VVS) is the most common cause of syncope in children. Recurrent syncope has been shown to adversely affect quality of patients' life,[1] although the prognosis is generally good. Clarifying the causative mechanism would be valuable in formulating an effective treatment regimen. Previous studies have evaluated the Bezold-Jarish reflex,[2-4] vascular endothelial dysfunction,[5-9] neuro-humoral mechanism,[10-13] and genetic factors[14-18] in the development of VVS, but the pathogenesis of VVS has remained unclear.

Previous studies revealed that excessive arterial flow-mediated dilation (FMD) was seen in children with VVS.[19] It has been reported that both the endothelium-dependent and endothelium-independent vasodilation are significantly increased in patients with VVS compared with healthy controls.[8] Shi et al reported that plasma nitric oxide might be involved in the development of VVS.[20] Raviele et al reported that patients with clinical episodes of VVS were particularly sensitive to the administration of organic nitrates such as nitroglycerin or isosorbide dinitrate.[21] Studies have revealed that endogenous hydrogen sulfide (H_2S) acts as a novel gasotransmitter[22,23] and could regulate vasorelaxant properties.[23-25] Zhang et al[9] reported that the plasma H_2S level in children with postural tachycardia syndrome was significantly higher than that of normal children. Because H_2S is an important gasotransmitter regulating vascular function, we attempted to identify whether there are any endogenous H_2S production abnormalities during the pathogenesis of VVS. Our present study was undertaken to test whether there are differences in erythrocyte H_2S production and explore its significance in the vascular mechanisms for VVS in children.

Methods

We enrolled 54 children in our study, of whom 27 (8 boys and 19 girls, mean age 11.3 ± 3.3 years) had VVS (VVS group) and 27 (17 boys and 10 girls; mean age 10.4 ± 1.8 years) served as controls (control group). Children with VVS were

BP	Blood pressure
FMD	Flow-mediated dilation
H_2S	Hydrogen sulfide
HR	Heart rate
HUTT	Head-up tilt test
VVS	Vasovagal syncope
VVS-CI	VVS-cardioinhibitory
VVS-MI	VVS-mixed inhibitory
VVS-VI	VVS-vasoinhibitory

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Supported by Major Basic Research Project of China (2013CB933801, 2011CB503904), National Twelfth Five-Year Plan for Science & Technology Support (2012BAI03B03), and Program for New Century Talent of Ministry of Education of China (NCET-11-0005). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2014.12.021

enrolled, after admission to the Department of Pediatrics, Peking University First Hospital, China. They were further divided into a VVS-vasoinhibitory (VVS-VI) subgroup, a VVS-cardioinhibitory (VVS-CI) subgroup, and a VVS-mixed inhibitory (VVS-MI) subgroup based upon their hemodynamics during head-up tilt test (HUTT).[26] The controls were considered healthy based on medical history, physical examination, and electrocardiogram. The study protocol adhered to the criteria from the Ethics Committee of Peking University First Hospital, Beijing, China. All participants' guardians were fully informed of the purpose and methods of the study, and then written informed consent was obtained.

The diagnosis of VVS[27] depends upon the following items: (1) usually a school-aged child; (2) a history of syncope; (3) the presence of predisposing factors in most of the patients; (4) positive haemodynamic and heart rate (HR) response during HUTT; and (5) exclusion of other causes of syncope. Positive responses during HUTT were as follows, children with syncopal episodes or presyncope with any of the following responses in HUTT were considered positive: (1) systolic blood pressure (BP) ≤80 mm Hg or diastolic BP ≤50 mm Hg or mean pressure decrease ≥25%; (2) HR <75 beats/min for 4-6 years old children; HR <65 beats/min for 7-8 years old children; HR <60 beats/min for those older than 8 years; (3) electrocardiogram showed sinus arrest, premature junctional contractions; and (4) atrioventricular block and cardiac arrest ≥3 seconds. The responses were classified as cardioinhibitory, vasoinhibitory, or mixed inhibitory.[28] The VVS-VI type was characterized by a significant BP decrease without obvious HR reduction, the VVS-CI type was characterized by a marked HR decrease without marked decrease in systolic pressure, and the VVS-MI type was characterized by both HR and BP decrease.

HUTT is as an objective method for diagnosing VVS.[27] The examination was carried out in a quiet and softly lit room. Patients were placed on a tilt table with a footboard at an angle of 60° (after 10 minutes in the supine position) until either a positive response appeared or the endpoint of at 45 minutes.[29,30] Dynamic electrocardiogram, HR and BP were monitored by a Dash 2000 Multi-Lead Physiological Monitor (General Electric Company, New York, New York). Baseline HR and BP in the supine for 10 minutes, and then continuous HR and BP after being tilted were recorded.

Patients with VVS were advised to avoid caffeine, high-fat foods, vitamin C, drugs, and exercise for 4-6 hours prior to testing because these might affect vasodilation. FMD by color Doppler vascular ultrasound was considered to be a noninvasive and reliable method of evaluating endothelial function. Study measurements were taken using a color Doppler Ultrasound system (Ultrasound Cardiograph, HP2500; Philips Healthcare, Andover, Massachusetts). The frequency of the transducer was 7.5 MHz.

With participants in the supine position, the transducer was placed on the skin about 5 cm above the antecubital fossa. The cuff of a mercury sphygmomanometer was placed around the forearm. A baseline image of the brachial artery was obtained to measure the diameter (baseline diameter) of the vascular lumen. The cuff was inflated to 40 mm Hg higher than the systolic BP causing an arterial occlusion. The inflation was maintained for 5 minutes. After the cuff was deflated, the brachial artery dilated temporarily. The image of the brachial artery was obtained, again at the same position within 2 minutes of deflation of the cuff. The maximum diameter of the vascular lumen during the dilation was recorded. The FMD was calculated by using the formula (maximum diameter − baseline diameter)/baseline diameter × 100%.

After 8 hours of fasting, blood was collected by venipuncture into a tube with heparin. The blood was centrifuged at 800× g for 7 minutes at 4°C, and the plasma and leukocyte cream were removed. The erythrocytes were washed twice with 0.1 mol/L phosphate buffered saline and centrifuged at 800× g for 5 minutes at 4°C. Cells were counted, and the erythrocytes were stored in the refrigerator until the assay was performed. 1×10^8 erythrocytes were lysed in 900 μL of ice-cold Tris-HCl (50 mmol/L, pH 7.4), and then ultrasound cracking was performed for 15 seconds. The erythrocyte lysate was transferred to a 25 mL Erlenmeyer flask and 100 μL of beta-mercaptopyruvate (Sigma, St. Louis, Missouri) was added, achieving a final concentration of 2 mmol/L. Central wells contained 0.5 mL of 1% sodium hydroxide as a trapping solution. The flasks were sealed quickly with a double layer of parafilm and incubated in a 37°C shaking water bath for 60 minutes, after which 0.5 mL of 20% trichloroacetic acid was added to stop the reaction. The flasks were sealed again and placed in the shaking water bath for another 60 minutes at 37°C to ensure complete trapping of H_2S. Erythrocytic H_2S production was measured by a sulphur-sensitive electrode. The H_2S production was expressed as unit nmol/min/10^8 erythrocytes.

Statistical Analyses

Statistical analyses were completed by SPSS 16.0 software (SPSS Inc, Chicago, Illinois). Continuous data are presented as mean ± SD. Comparisons between the VVS group and the control group were performed using an independent-sample *t* test. Comparison among the VVS-VI, VVS-CI, and VVS-MI subgroups was performed using ANOVA. Linear correlation was used to analyze the correlation between erythrocyte H_2S and FMD. $P < .05$ was considered statistically significant.

Results

There were no statistical differences between the VVS group and the control group in age, sex, height, and weight. There were no statistical differences in the frequency of syncope, duration of illness, positive response appearance time in HUTT, and positive response time in HUTT among VVS-VI, VVS-CI, and VVS-MI subgroups ($P > .05$).

Erythrocyte H_2S production in the VVS group was significantly higher than that of control group (32.9 ± 12.5 vs 15.0 ± 4.0 nmol/min/10^8 erythrocytes, $P < .001$)