Fibrinolytic System in Adolescents: Response to Venous Occlusion Stress Tests

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ABSTRACT

Recent studies indicate that the incidence of thromboembolic events is increasing as a secondary complication in children with serious underlying diseases. The mechanism to eliminate these thrombi *via* the thrombolytic system in children is unknown. The baseline fibrinolytic system is age dependent, with significant variation between children and adults. Adult studies would suggest that the fibrinolytic response to venous occlusion has more clinical relevance than the baseline fibrinolytic system. The aim of this study was to determine whether the fibrinolytic response to venous occlusion stress testing in healthy adolescents differs from the response in healthy adults. Healthy adolescents (13–18 y) from a school population and normal adults were recruited. Pre- and postvenous occlusion blood samples were collected using standard techniques. Plasma tissue plasminogen activator, plasminogen activator inhibitor-1, plasminogen, α_2-antiplasmin, α_2-macroglubulin, D-dimers, euglobulin lysis time, and fibrinogen were measured on each sample. Adolescents had significantly decreased tissue plasminogen activator antigen levels and increased plasminogen activator inhibitor-1 activity levels after venous occlusion, resulting in significantly prolonged euglobulin lysis times. The results of our study confirm developmental differences in the fibrinolytic response to venous occlusion stress testing. The age-related differences in fibrinolytic response to venous occlusion of younger children and the significance of these differences on the pathophysiology of thromboembolic events in children require further studies. (*Pediatr Res* 53: 333–337, 2003)

Abbreviations
TE, thromboembolic events
tPA, tissue plasminogen activator
PAI, plasminogen activator inhibitor
α_2-AP, α_2-antiplasmin
α_2-M, α_2-macroglobulin
ELT, euglobulin clot lysis time
CI, confidence interval

Thromboembolic events occur relatively rarely during childhood, even in the presence of acquired or congenital risk factors (1, 2). In contrast, TE occur in approximately 5% of the adult population, and may be idiopathic, or secondary to congenital or acquired disorders. The profound difference in incidence of TE in children suggests that there are protective mechanisms in place in the young. Recent studies have focused on physiologic differences in the coagulation system and vessel wall in the young and the role that these physiologic differences may have in protecting children from TE. These studies have shown that there is a decreased capacity to generate thrombin, a key enzyme in coagulation, enhanced regulation of thrombin by inhibitors such as α_2-M, a circulating anticoagulant with antithrombin activity at birth, and increased amounts of proteoglycans with antithrombin activity in the vessel wall of the young (3–6).

Although extensive information on the coagulation system in the young is available, specifically on the regulation of thrombin, there is little information on the fibrinolytic system and its potential role in preventing TE during childhood. At birth, all components of the fibrinolytic system are present but with differing plasma concentrations of key components, and evidence of significant, transient activation (7). Throughout childhood, the ratio of tPA to PAI-1 is reversed compared with adults, suggesting that the fibrinolytic system may be relatively suppressed and may not provide increased protection from TE in the young (1).

Although the incidence of TE in children is reduced compared with adults, recent population-based studies in Canadian children have shown that the incidence of TE is increasing as a secondary complication in children with serious underlying diseases such as cancer and congenital heart disease (2). Persistent thrombi result in obstruction of the venous system with the associated risk of recurrent TE and postphlebitic syndrome. To date, there is no information in the literature on the developmental differences in the overall fibrinolytic capacity of children using tests sensitive to *in vivo* responses such as venous occlusion. Therefore, the objective of the current study was assess the *in vivo* response of the fibrinolytic system to venous occlusion in healthy adolescents. Adolescents are a particularly relevant population to assess as they are the second most common age group in which TE occur in pediatric patients (2).

METHODS

Study Population

Healthy adolescents on no medications (including the oral contraceptive pill) between 13 and 18 y of age attending a school in Oakville, Ontario, Canada, were approached to participate in the study. Informed consent was obtained from both the adolescents and their parents before participation. Healthy adults aged 20–50 y on no medications (including the oral contraceptive pill) were used as controls. The study was approved by the ethics review board at Chedoke McMaster University, Hamilton, Ontario, Canada.

Sample Collection

Subjects and controls fasted from 0000 h before the morning of testing. All blood collections were performed between 0800 and 1000 h. On arrival, patients had local anesthetic cream applied to both antecubital fossae. After 15 min resting supine, pre-stress test samples were collected *via* a single, clean venipuncture from the antecubital fossa of a non-occluded arm. Tourniquet use was avoided or kept to a minimum (<1 min). Ten milliliters of blood were collected into 3.2% sodium citrate (nine parts blood: one part citrate) for tPA antigenic assay, PAI-1 functional and antigenic assays, plasminogen, α_2-AP, α_2-M, D-dimer, ELT, and fibrinogen. A further sample was collected into acidified sodium citrate tubes (pH 4.3) (Stabilyte tubes, American Diagnostica, Greenwich, CT, U.S.A.) for tPA functional assays, and 0.5 mL into EDTA for hematocrit determination. A blood pressure cuff appropriate to the size of the subject was then inflated to mid-systolic/diastolic pressure for 10 min. Postocclusion samples, identical to the preocclusion samples, were obtained from the occluded arm before deflation of the blood pressure cuff. Blood samples were placed immediately into wet ice and then centrifuged at $1700 \times g$ for 15 min at 4°C within 20 min of collection. Platelet poor plasma was aliquoted and frozen at -70°C until time of assay. Postocclusion values were corrected for hematocrit changes using the following correction factor (F): $F = H_1(1 - Bd \times H_2)/H_2(1 - Bd \times H_1)$, where H_1 = hematocrit before occlusion, H_2 =

hematocrit after occlusion, and $Bd = 0.9$ when blood and anticoagulant are mixed in a ratio of 9:1 (8).

Assays

The following assays were performed on all pre- and postvenous occlusion samples: tPA antigen (Imubind, tPA, American Diagnostica); tPA activity (Spectrolyse tPA/PAI, American Diagnostica); PAI-1 antigen (Imubind, PAI-1, American Diagnostica); PAI-1 activity (Spectrolyse PAI, American Diagnostica); plasminogen antigen (ELISA: Imuclone, American Diagnostica); plasminogen activity (Actichrome PLG, American Diagnostica), and α_2-AP activity (Actichrome American Diagnostica) using an Automated Coagulation Laboratory (ACL, Milan, Italy); α_2-M antigen by radial immunodiffusion using commercially available antibody (Cedarlane Laboratories Limited, Hornby, Ontario, Canada); D-dimer (Dimertest Gold EIA, American Diagnostica); ELT [modified from Bucknell (9)]; and fibrinogen (functional) by semiautomated Clauss method (ST4, Diagnostica Stago, Asnières, France). Hematocrits were measured on a Coulter STK-S (Coulter Canada, Burlington, ON, Canada). The D-dimer assay was chosen over fibrinogen degradation products (FDP) as D-dimers are specific to plasmin degradation of cross-linked fibrin only, whereas FDP detect plasmin proteolysis of either fibrinogen or fibrin (10).

tPA/PAI-1 ratios. The ratio represents a measure of the fibrinolytic capacity in patients by assessing the relative relationship of plasma levels of tPA to plasma levels of tPA's main inhibitor, PAI-1. An increased ratio (*i.e.* increased tPA and/or decreased PAI-1) would be characterized as a hyper fibrinolytic state. The converse, a decreased ratio (*i.e.* decreased/normal tPA and increased PAI-1), would be characterized as a hypofibrinolytic state. The tPA/PAI-1 ratios were calculated using the antigen levels for both proteins.

Statistical Analysis

The mean values for pre- and postocclusion results with 95% CI were calculated for each variable. Preocclusion and postocclusion were compared for adult controls and adolescents using t test. Between-gender differences were assessed using one-way ANOVA with posthoc comparisons. p Values < 0.05 were considered statistically significant.

RESULTS

Samples were collected from 15 adolescent girls, mean age 15.7 y, (range, 13–17 y) and 12 adolescent boys, mean age 16.4 y (range, 15–18 y). In one girl, a postocclusion sample was not obtained because of difficult venous access. All teenage girls were postmenarche on history (formal Tanner staging was not performed). Mean ages of the female and male adult controls were 27 ($n = 10$) and 29.4 y ($n = 10$), respectively.

There were no differences between male and female results within adolescents or control groups for the majority of the analyses. Hence, results are described for all adolescents and all adult controls, with any gender differences subsequently identified. Overall pre- and post-occlusion values ($\pm$ 95% CI)

for adolescents and adults are shown in Tables 1 and 2, respectively. Mean tPA/PAI-1 ratios were decreased in adolescents compared with adults both pre-occlusion (adolescents: 0.29, adults: 0.44, $p = 0.05$) and post-occlusion (adolescents: 0.66, adults: 1.12, $p = 0.03$). Significant gender differences were present for PAI-1 activity (Table 3). Mean values of PAI-1 activity pre- and post-occlusion were significantly increased in teenage males compared with teenage females, adult males, and adult females (pre-occlusion: $p = 0.001$, post-occlusion: $p = 0.004$). The relationship of PAI-I antigen levels were consistent with the PAI-1 activity levels but the trend did not reach statistical significance (data not shown). Mean values of ELT post-occlusion were significantly increased in adolescents compared with adults ($p = 0.0001$) and there was no difference based on gender (Table 4).

DISCUSSION

Our understanding of developmental hemostasis has rapidly expanded during the last two decades, with multiple studies confirming the dynamic process of maturation of the coagulation system during childhood. The altered physiologic status of the coagulation system in the young has a profound influence on the incidence of TE and on the response to anticoagulants (11, 12). In contrast to the coagulation system, relatively little is known about the maturation of the fibrinolytic system during childhood other than baseline plasma concentrations of fibrinolytic proteins. The available information suggests that the fibrinolytic system may be suppressed during childhood. However, assessments of the fibrinolytic capacity by a test sensitive to *in vivo* fibrinolytic response, such as a venous stress testing, has not previously been systematically assessed in adolescents and compared with adults. The results of this study demonstrate, using venous occlusion stress testing, that fibrinolytic response to venous occlusion is reduced in adolescents compared with adults.

The capacity to thrombolyze fibrin clots *in vivo* is likely influenced by many components of the fibrinolytic system, but particularly tPA and PAI-1 (13). Under basal conditions, tPA circulates mainly as a complex with PAI-1 with low levels of free rtPA (13–16). Synthesis of both tPA and PAI-1 occurs in the vascular endothelial cells and, in the resting state, tPA is present on the luminal surface of the endothelium (17–19). Plasmin generation can be increased by both up-regulation of tPA production by endothelial cells and secretion of tPA into the vascular system (20, 21). The ELT is an *in vitro* measure of the fibrinolytic potential of plasma. Historically, the ELT is the most reliable global assessment of fibrinolytic function, and remains the gold standard today, although the sensitivity of the ELT to some factors influencing fibrinolytic potential, such as thrombin activatable fibrinolysis inhibitor, is questionable.

Although the majority of components of the fibrinolytic system are present during infancy and childhood, there are some important developmental differences. Although plasma concentrations of tPA, PAI-1, and α_2-M are significantly increased at birth compared with adult values, plasma concentrations of plasminogen and α_2−AP are decreased to 50% and 80% of adult values, respectively (1). The decreased plasma concentrations of plasminogen result in reduced thrombolysis of fibrin clots *in vitro*. (22) After birth, plasminogen and α_2−AP are similar to adult values by 6 mo of life (23). However, the ratio of tPA to PAI-1 is reversed throughout childhood, suggesting that the fibrinolytic system is suppressed compared with adults (1). Significant differences in the plasma concentrations of α_2-M between children and adults persist until late adolescent years, as confirmed again in this study.

We have assessed fibrinolytic response to venous occlusion in healthy adolescents. Adolescents are particularly relevant as, after infants, this is the most common age at which thrombosis in children occurs (2). Assessment of fibrinolytic response to venous occlusion in infants will require different methodology because venous occlusion is not well tolerated by small children. The adolescents in our study tolerated the procedure very well, with minimal discomfort and arm movement, however, the youngest children in the study required active distraction after 5 min of occlusion.

The results of our study show reduced fibrinolytic response to venous occlusion in adolescents compared with adults because of decreased release of tPA, and increased levels of PAI-1, resulting in both decreased tPA/PAI-1 ratios and prolongation of the ELT. In adults, impaired fibrinolytic response to venous occlusion, specifically reduced release of tPA and increased release of PAI-1, has been implicated in the etiology

Table 1. *Values before venous occlusion*

Parameter	Adolescents ($n = 26$)		Adults ($n = 19$)		p Value*
	No.	Value	No.	Value	
tPA activity (U/mL)	25	1.89 (0.54)	12	1.95 (0.50)	NS
tPA antigen (ng/mL)	26	5.35 (1.28)	19	6.95 (2.22)	NS
PAI-1 activity (U/mL)	25	11.92 (3.78)	19	6.58 (2.06)	0.02
PAI-1 antigen (ng/mL)	26	24.16 (6.88)	18	23.44 (10.30)	NS
Plasminogen activity (U/mL)	26	1.10 (0.08)	19	1.17 (0.04)	NS
Plasminogen antigen (μg/mL)	26	0.24 (0.02)	19	0.25 (0.02)	NS
α_2-AP (U/mL)	26	1.02 (0.04)	19	1.00 (0.06)	NS
α_2-M (U/mL)	26	1.27 (0.08)	19	0.96 (0.12)	<0.0001
D-dimer (ng/mL)	25	25.00 (5.14)	18	25.00 (7.54)	NS
ELT (h)	26	5.00 (0.30)	19	4.50 (0.50)	NS
Fibrinogen (g/L)	26	2.34 (0.10)	19	2.64 (0.22)	NS

Values given are mean $\pm$ 95% confidence interval.

* Analyzed by one-way ANOVA.

Table 2. *Values after venous occlusion*

Parameter	Adolescents (n = 26)		Adults (n = 19)		p Value*
	No.	Value	No.	Value	
tPA activity (U/mL)	25	2.08 (0.36)	12	2.42 (0.50)	NS
tPA antigen (ng/mL)	26	9.91 (1.60)	19	14.22 (2.48)	0.004
PAI-1 activity (U/mL)	25	9.43 (2.96)	19	5.19 (0.42)	0.02
PAI-1 antigen (ng/mL)	26	24.02 (8.00)	18	24.88 (12.42)	NS
Plasminogen activity (U/mL)	26	1.06 (0.1)	19	1.15 (0.08)	NS
Plasminogen antigen (μg/mL)	26	0.20 (0.01)	19	0.21 (0.02)	NS
α_2-AP (U/mL)	26	0.93 (0.06)	19	0.87 (0.06)	NS
α_2-M (U/mL)	26	1.20 (0.08)	19	0.92 (0.10)	0.0004
D-dimer (ng/mL)	25	17.00 (4.12)	18	20.00 (7.74)	NS
ELT (h)	26	3.10 (0.40)	19	1.80 (0.50)	0.0001
Fibrinogen (g/L)	26	2.16 (0.14)	19	2.25 (0.30)	NS

Values are given as mean ± 95% confidence interval.
* Analyzed by one-way ANOVA.

Table 3. *Pre- and post-occlusion results of PAI-1 activity expressed as mean (±95% confidence interval)*

PAI-1 activity (U/mL)	Adolescents		Adults		p Value*
	Female	Male	Female	Male	
Pre-occlusion	7.40 (2.26)	16.82 (6.48)†	5.66 (2.54)	7.40 (3.24)	0.001†
Post-occlusion	6.32 (1.80)	12.79 (5.30)†	4.30 (1.84)	5.99 (2.12)	0.004†

* Analyzed by one-way ANOVA.
† Adolescent males had significantly increased PAI-1 activity levels when compared with female adolescents, female adults, and male adults.

Table 4. *Pre- and postocclusion results of ELT expressed as mean (±95% confidence interval)*

ELT (h)	Children		Adults		p Value*
	Female	Male	Female	Male	
Pre-occlusion	5.2 (0.30)	4.7 (0.50)	4.2 (0.80)	4.8 (0.60)	NS
Post-occlusion	3.0 (0.50)†	3.2 (0.60)†	1.7 (0.80)	2.0 (0.60)	0.002

* Analyzed by one-way ANOVA.
† Adolescents had significantly longer ELT postocclusion when compared with adults. There was no effect of gender.

of TE (24–27), particularly postoperative TE (24–33). The majority of TE in children are associated with central venous lines, which likely cause a degree of endothelial disruption, especially at the time of central venous line insertion. The reduced fibrinolytic response to venous occlusion reported in our study may imply that the clinical course of TE in adolescents have limited resolution with time.

CONCLUSION

In summary, our study concludes that adolescents have a decreased fibrinolytic capacity, as defined by the fibrinolytic response to venous occlusion, when compared with adults. The results are consistent with the previously documented baseline fibrinolytic parameters that suggested reduced fibrinolysis in adolescents. Further studies will be required to determine the magnitude of likely differences in younger children. The data reported by this study provide a baseline that will enable further investigation on the role of reduced fibrinolytic capacity in TE in children.

REFERENCES

1. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L 1992 Maturation of the hemostatic system during childhood. Blood 80:1998–2005
2. Andrew M, David M, Adams M, Ali K, Anderson R, Barnard D, Bernstein M, Brisson L, Cairney B, DeSai D 1994 Venous thromboembolic complications (VTE) in children: first analyses of the Canadian Registry of VTE. Blood 83:1251–1257
3. Massicotte P, Leaker M, Marzinotto V, Adams M, Freedom R, Williams W, Vegh P, Berry L, Shah B, Andrew M 1998 Enhanced thrombin regulation during warfarin therapy in children compared to adults. Thromb Haemost 80:570–574
4. Ling X, Delorme M, Berry L, Ofosu F, Mitchell L, Paes B, Andrew M 1995 alpha 2-Macroglobulin remains as important as antithrombin III for thrombin regulation in cord plasma in the presence of endothelial cell surfaces. Pediatr Res 37:373–378
5. Andrew M, Mitchell L, Berry L, Paes B, Delorme M, Ofosu F, Burrows R, Khambalia B 1992 An anticoagulant dermatan sulfate proteoglycan circulates in the pregnant woman and her fetus. J Clin Invest 89:321–326
6. Nitschmann E, Berry L, Bridge S, Hatton MW, Richardson M, Monagle P, Chan AKC, Andrew M 1998 Morphological and biochemical features affecting the antithrombotic properties of the aorta in adult rabbits and rabbit pups. Thromb Haemost 79:1034–1040
7. Pinacho A, Paramo JA, Ezcurdia M, Rocha E 1995 Evaluation of the fibrinolytic system in full term neonates. Int J Clin Lab Res 25:149–152
8. Keber D 1983 On the use of different correction factors for hemoconcentration. Thromb Haemost 49:245

9. Bucknell M 1958 The effect of citrate on euglobin methods of estimating fibrinolytic activity. J Clin Pathol 11:403–405
10. Eisenberg PR, Jaffe AS, Stump DC, Collen D, Bovill EG 1990 Validity of enzyme-linked immunosorbent assays of cross-linked fibrin degradation products as a measure of clot lysis. Circulation 82:1159–1168
11. Andrew M 1998 Developmental hemostasis: relevance to newborns and infants. In: Nathan DG, Orkin SH (eds) Hematology of Infancy and Childhood, 5th Ed. WB Saunders, Philadelphia, pp 114–157
12. Andrew M 1992 Anticoagulation and thrombolysis in children. Tex Heart Inst J 19:168–77
13. Takahashi H, Tatewaki W, Wada K, Niwano H, Hanano M, Shibata A 1991 Plasmin generation and fibrin(ogen)olysis following desmopressin infusion. Am J Hematol 36:255–258
14. Collen D 1980 On the regulation and control of fibrinolysis. Edward Kowalski Memorial Lecture. Thromb Haemost 43:77–89
15. Wun TC, Capuano A 1987 Initiation and regulation of fibrinolysis in human plasma at the plasminogen activator level. Blood 69:1354–1362
16. Collen D, Lijnen HR 1991 Basic and clinical aspects of fibrinolysis and thrombolysis. Blood 78:3114–3124
17. Van Hinsberg VW, Sprengers ED, Kooistra T 1987 Effect of thrombin on the production of plasminogen activators and PA inhibitor-1 by human foreskin micro-vascular endothelial cells. Thromb Haemost 57:148–153
18. Cheng XF, Brohlin M, Pohl G, Back O, Wallen P 1995 Binding of tissue plasminogen activator to endothelial cells. The effect on functional properties. Localization of a ligand in the B-chain of tPA. Thromb Res 77:149–164
19. Smokovitis A 1978 Regions of constantly increased plasminogen activator activity along the intima of the normal aorta. Haemostasis 7:303–319
20. Davidson JF, Walker ID 1987 Assessment of the fibrinolytic system. In: Bloom AL, Thomas DP (eds) Haemostasis and Thrombosis, 2nd Ed. Churchill Livingstone, New York, pp 953–966
21. Rijken DC, Hoylaerts M, Collen D 1982 Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. J Biol Chem 257:2920–2925
22. Andrew M, Brooker L, Leaker M, Paes B, Weitz J 1992 Fibrin clot lysis by thrombolytic agents is impaired in newborns due to a low plasminogen concentration. Thromb Haemost 68:325–330
23. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, Powers P 1987 Development of the human coagulation system in the full-term infant. Blood 70:165–172
24. Levi M, Lensing AWA, Buller HR, Prandoni P, Dooijewaard G, Cuppini S, ten Cate JW 1991 Deep vein thrombosis and fibrinolysis. Thromb Haemost 66:426–429
25. Jorgensen M, Bonnevie-Nielsen V 1987 Increased concentration of the fast-acting plasminogen activator inhibitor in plasma associated with familial venous thrombosis. Br J Haematol 65:175–180
26. Jorgensen M, Mortensen JZ, Madsen AG, Thorsen S, Jacobsen B 1982 A family with reduced plasminogen activator activity in blood associated with recurrent venous thrombosis. Scand J Haematol 29:217–223
27. Stegnar M, Peternel P, Keber D, Vene N 1992 Poor fibrinolytic response to venous occlusion by different criteria in patients with deep venous thrombosis. Thromb Res 64:445–453
28. Nilsson I, Ljungner H, Tengborn L 1985 Two different mechanisms in patients with venous thrombosis and defective fibrinolysis: low concentration of plasminogen activator or increased concentration of plasminogen activator inhibitor. BMJ 290:1453–1456
29. Lowe GDO 1997 Prediction of postoperative deep vein thrombosis. Thromb Haemost 78:47–52
30. Aberg M, Nilsson I 1978 Fibrinolytic activity of the vein wall after surgery. Br J Surg 65:259–262
31. Kluft C, Jie AF, Lowe GD, Blamey SL, Forbes CD 1986 Association between postoperative hyper-response in t-PA inhibition and deep vein thrombosis. Thromb Haemost 56:107
32. Sue-Ling H, Johnston D, Verheijen J, Kluft C, Philips P, Davies J 1987 Indicators of depressed fibrinolytic activity in pre-operative prediction of deep venous thrombosis. Br J Surg 74:275–278
33. Macintyre IMC, Webber RG, Crispin JR, Jones DR, Wood JK, Allan NC, Prescott RJ, Ruckley CV 1976 Plasma fibrinolysis and postoperative deep vein thrombosis. Br J Surg 63:694–697