Understanding hemostasis and thrombosis : The lesson learnt from natural models

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Under physiologic conditions, a hemostatic balance is achieved through the effects of natural procoagulant and anticoagulant factors which, in equilibrium with each other, provide hemostasis at the sites of vascular injury. Abnormalities of these hemostasis factors result in a tendency towards hemorrhagic or thrombotic events. In this review the influence of inherited prothrombotic risk factors—especially the frequent factor V Leiden and prothrombin gene mutations—on normal and abnormal hemostasis is analyzed from an evolutionary point of view. The effect of inherited bleeding disorders on the development of thrombotic or atherosclerotic processes is also discussed.

Key words: thrombophilia, bleeding disorders, venous thromboembolism, atherothrombosis

Introduction

The hemostatic balance is the result of a delicate equilibrium between procoagulant and anticoagulant factors that interact with each other to ensure effective hemostasis at the sites of vascular injury. However, structural abnormalities and/or changes in the levels of the different components of the hemostatic system can result in disturbances that may cause bleeding or thrombotic disorders. Mechanisms of hemorrhage include defects of coagulation factors (the hemophilias) and platelet function (Glanzmann thrombasthenia, Bernard-Soulier syndrome and von Willebrand disease), while mechanisms of thrombosis include the deficiency of naturally occurring anticoagulants (antithrombin, protein C and protein S), increased plasma levels of

coagulation factors (fibrinogen, factor VIII, von Willebrand factor) and gain-of-function of coagulation factors (factor V Leiden, and prothrombin gene mutations)¹⁾⁻³⁾. Among the inherited prothrombotic risk factors, the factor V Leiden mutation, which causes resistance to activated protein C (APC) due to the substitution of adenine to guanine at nucleotide 1691 at the cleavage site (Arg506) of activated factor V by ACP, is present in heterozygosity in about 5 percent of Caucasians, but is absent in Africans, Chinese, Japanese and native North and South Americans⁴⁾. Haplotype analyses suggest a single origin for the mutation, which is estimated to have arisen approximately 22,000 years ago well after the evolutionary separation of non-Africans from Africans (which occurred approximately 100,000 years ago) and of

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Caucasoid from Mongoloid populations (which occurred approximately 40,000 years ago)⁵⁾. At more or less the same time of mankind evolution a single genetic origin occurred for another common gain-of-function mutation, the G20210A mutation in the prothrombin gene. Among Caucasians, the prevalence of factor II G20210A heterozygotes is 1 to 6 percent, whereas in non-Caucasian populations the mutation is very rare or absent, as for factor V Leiden⁶⁾. Both mutations are associated with an increased risk of venous thromboembolism^{7,8)}, and a meta-analysis indicates that they also confer a mildly increased risk of arterial thromboembolic disease⁹⁾. However, due to their prevalence and persistence through millennia among Caucasians, it is unlikely that these mutations have only deleterious effects on health, because evolutionary selection pressure would have already led to their elimination from the human genome¹⁰⁾. Why are thrombophilic mutations so frequent? Do they really give “bad blood”¹⁰⁾ or do they afford evolutionary advantages, as sickle cell anemia, thalassemia and glucose-6-phosphate dehydrogenase deficiency do in terms of protection from malaria? And, on the other side of the hemostatic balance, does the hypocoagulability underlying inherited bleeding disorders confer protection against thrombosis and/or atherosclerosis?

The hemostatic balance : thrombosis

There is increasing evidence that gain-of-function prothrombotic mutations modulate the hemostatic balance by decreasing the amount of bleeding in a number of physiological and clinical situations. In a retrospective study, Lindqvist et al. compared the peri-partum blood loss in women with or without factor V Leiden¹¹⁾.

Significantly lower blood losses (318 ml vs. 380 ml, $P=0.018$) and a smaller difference between pre- and post-partum hemoglobin (0.30 g/dl vs. 0.80 g/dl, $P=0.02$) were found in carriers of the mutation. Subsequently, the same authors demonstrated that carriership of factor V Leiden was associated with higher hemoglobin and serum ferritin and reduced menstrual blood loss¹²⁾, suggesting that the gain-of-function mutation might have conferred to women an evolutionary advantage by protecting them against bleeding at parturition and menses.

Another possible pregnancy-related evolutionary benefit was surmised by Gopel et al.¹³⁾, who investigated 102 mother-child pairs who successfully underwent *in-vitro* fertilization and found that the first embryo implantation was successful in 90 percent of mother-child pairs carrying the factor V Leiden, compared with 49 percent ($P=0.018$) in factor V Leiden negative pairs. Furthermore, the median number of unsuccessful transfers of embryos was smaller in pairs positive for the mutation than in those who were negative ($P=0.02$) suggesting that improved implantation rate is an important advantage provided by hypercoagulability associated with the factor V Leiden mutation.

Other studies have analyzed the influence of gain-of-function mutations of coagulation factors on hemorrhage in conditions other than parturition. Corral et al.¹⁴⁾ conducted a study on 201 patients with spontaneous intracranial hemorrhage and a corresponding number of controls and found that the presence of factor V Leiden reduced the risk of intracranial hemorrhage by 5-fold (1% vs. 4.9%, $P=0.019$). In addition, the frequency of the G20210A prothrombin mutation in patients was half that in controls, while carriers of the -323 insertion in the factor VII gene promoter, associated with

lower factor VII levels and hence with a mild hypocoagulability, had a 1.5-fold increased risk of intracranial hemorrhage. A more recent study, carried out in 103 adults with subarachnoid hemorrhage¹⁵⁾ suggested that the platelet glycoprotein IIIa polymorphism HPA-1 (PIA), which causes platelet hyper-reactivity, has a protective role. By contrast, Navarro-Nunez et al.¹⁶⁾ observed that the β 1-tubulin platelet polymorphism Q43P, by causing a lower reactivity of platelets, protects against thrombotic disorders but increases the risk of intracerebral hemorrhage. Gopel et al.¹⁷⁾ analyzed 305 preterm, very low birth weight infants and found a low prevalence of intraventricular hemorrhage in those carrying the factor V Leiden or prothrombin G20210A mutation (OR 0.11, CI 0.02–0.5), suggesting that a heightened coagulability due to gain-of-function mutations helps to prevent the occurrence of intraventricular bleeding in very low birth weight infants. However, other studies on the influence of these mutations in very low birth weight infants produced conflicting results¹⁸⁾¹⁹⁾. An additional piece of evidence that these mutations decrease bleeding stems from a study conducted on 517 patients undergoing cardiac surgery, including 26 heterozygous factor V Leiden carriers²⁰⁾. Donahue et al.²⁰⁾ demonstrated that the gain-of-function mutation protects from blood loss and reduces transfusion requirements after cardiac surgery, the epitome of surgical operations associated with major blood loss.

Primitive men were hunters but were also preys of animals and of their peers. Bleeding and infections were conditions that primitive men had to tackle as consequences of the wounds suffered in their “fight and flight”. Several studies have evaluated whether or not gain-of-function mutations offered protection from infections. The

effect of the factor V Leiden mutation on survival in sepsis was first studied by Kerlin et al. in an endotoxin mouse model and in a large human clinical trial, the PROWESS study (recombinant human activated Protein C Worldwide Evaluation in Severe Sepsis)²¹⁾. There was a lower mortality in heterozygous factor V Leiden mice and a lower 28-day mortality in patients with heterozygous factor V Leiden than in those without (13.9% vs. 27.9%, $P=0.013$), suggesting that the beneficial effect of the gain-of-function mutation might be related to the increase of the anticoagulant effect of protein C activation by enhanced thrombin formation. However, the protective effect of factor V Leiden on sepsis was not confirmed in two subsequent studies. Yan and Nelson²²⁾ combined the results of PROWESS and ENHANCE (Extended Evaluation of Recombinant Human APC) recruiting 3894 adults with severe sepsis and found no statistically significant difference in the 28-day mortality between heterozygous factor V Leiden carriers and non-carriers (20.3% vs. 24.9% ; risk ratio 0.82, CI 0.57–1.17). In a population-based study (the Copenhagen City Heart Study involving 1093 patients hospitalized for infections), Nelson et al.²³⁾ found that the 28-day mortality for sepsis was actually increased in carriers of factor V Leiden.

The hemostatic balance : bleeding

In order to provide further support to the evolutionary model of the hemostatic balance, some authors have evaluated whether or not gain-of-function mutations provide selective advantages in patients with inherited bleeding disorders by modulating the clinical phenotype²⁴⁾. It is well known that there is a great variability in the pattern and age of onset of bleeding among patients with severe hemophilia and that a subset

of patients who have severe hemophilia on laboratory testing (factor level less than 1% of normal) has a mild clinical phenotype²⁵⁾.

Why do these differences occur and what is their explanation? Besides the possibility that the type of mutation within the factor VIII gene may influence the clinical severity of hemophilia²⁶⁾²⁷⁾ it has recently been suggested that prothrombotic gene mutations modulate clinical phenotype²⁸⁾. In 1996, Nichols et al.²⁹⁾ studied the factor V Leiden mutation in two sets of hemophiliacs who had identical factor VIII gene mutations but different disease severity and suggested that co-inheritance of factor V Leiden conferred a clinical benefit. Ghosh et al.³⁰⁾ described 11 patients with a milder clinical presentation of hemophilia A and found that 8 of them had one or more inherited prothrombotic factors (i.e., protein C, protein S and antithrombin deficiencies, heterozygosity for factor V Leiden and for methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T). Ettinghausen et al.³¹⁾ evaluated 124 previously untreated children with hemophilia A and found that those carrying the factor V Leiden mutation had their first bleeding episode at a later time in life than did non-carriers. These results were confirmed by Nowak-Göttl et al.³²⁾ and by Lee et al.³³⁾. Moreover, the latter group showed that coagulation factor usage for treatment of bleeding was lower in carriers of the Leiden mutation than in those without.

The molecular mechanism by which factor V Leiden affects the hemophilia phenotype seems to be related to an increase in thrombin generation by reducing thrombin down-regulation through the APC pathway. In an *in vitro* model, van't Veer et al.³⁴⁾ demonstrated that thrombin formation was increased in the presence of factor V Leiden when all the factors of the protein C pathway were present in the reaction. Activated

factor V has a cofactor function in the proteolysis of factor VIII by APC. However, proteolytic degradation is considerably smaller in the presence of activated factor V (FVa). The inactivation of FVa Leiden is slower than that of wild-type FVa. Thus, FVa Leiden may protect factor VIII against APC-induced inactivation.

In contrast, other groups failed to observe a decrease in bleeding in hemophiliacs carrying factor V Leiden³⁵⁾⁻³⁷⁾. Arbini et al.³⁵⁾ evaluated 21 patients with severe hemophilia A or B and a milder clinical phenotype and found that only one of them was a carrier of factor V Leiden mutation. Similarly, Arruda et al.³⁶⁾ found that 3 of 113 patients with hemophilia of different severity had co-inherited the factor V Leiden mutation but failed to detect a difference in the frequency of bleeding episodes or need for replacement therapy between patients with or without the mutation. Finally, Araujo et al.³⁷⁾ screened 37 patients with hereditary disorders for various prothrombotic mutations but found no protective effect on clinical bleeding by any of the mutations. The modifying role of prothrombotic gene mutations was also evaluated in patients with inherited hemorrhagic diseases other than hemophilia. For instance, Castoldi et al. reported that the coinheritance of FV Leiden in patients homozygous for the FVII Lazio mutation was associated with a milder bleeding phenotype³⁸⁾. On the whole, these data are quite contradicting and inconclusive, and fail to establish convincingly that a mild clinical expression of a severe bleeding disorder is explained by the presence of the factor V Leiden mutation or other prothrombotic factors.

A number of studies analyzed the opposite side of the hemostatic balance, i.e. whether or not the presence of a hemostatic defect provides protection from thrombosis. Cardiovascular events due to thrombosis are uncommon in

hemophiliacs. In a long-term follow-up of Dutch hemophiliacs, Triemstra et al.³⁹⁾ found that the risk of fatal ischemic heart disease in this population was 80% lower than expected, suggesting that severe and moderately severe hemophilia offers protection against ischemic heart disease³⁹⁾. These data have been confirmed by the recent observation that a reduction of coagulation factors VIII and IX to approximately 50 percent of normal in female carriers of hemophilia A and B protected them from ischemic heart disease⁴⁰⁾. Kiechl and Willeit⁴¹⁾ suggested that iron depletion associated with recurrent bleeding, could explain the decreased risk of thrombosis.

On the other hand, Salomon et al.⁴²⁾ found that severe factor XI deficiency, unlike factor VIII and IX deficiency, did not confer protection against acute myocardial infarction. Ultrasonographically measured intima-media thickness in the carotid and femoral arteries of patients with inherited bleeding disorders (hemophilia and von Willebrand disease) was not different from that in healthy controls, indicating that hereditary conditions characterized by decreased coagulability have no relevant protective effect against atherosclerosis⁴³⁾. Further studies on patients with inherited defects of primary hemostasis have confirmed these findings using the same method. A study conducted on 7 patients with Glanzmann thrombasthenia⁴⁴⁾ found no significant differences in intima-media thickness and atherosclerotic plaques between patients and controls. Similar results were found in a recent multicenter study conducted in 47 patients with type 3 von Willebrand disease the most severe form due to marked defects of the platelet adhesive glycoprotein von Willebrand factor⁴⁵⁾.

In all, these results, along with the findings of a

decreased thrombotic tendency in animal models of von Willebrand disease and Bernard-Soulier syndrome⁴⁶⁾⁴⁷⁾, corroborate the hypothesis that primary hemostasis (i.e., platelets and von Willebrand factor) does not play a role in the development and extent of the atherosclerotic process, and that the putative protection from atherothrombotic cardiovascular disease observed in epidemiological studies carried out in patients with hemophilia is perhaps due to protection from thrombus formation on atherosclerotic plaques⁴⁸⁾.

Learning points

The hypothesis that evolutionary advantages have maintained gain-of-function prothrombotic mutations that developed approximately 22,000 years ago in the pre-Neolithic man is very intriguing. In the primitive man, bleeding from wounds and parturition was a frequent and prominent event in daily life. The same men were lean and physically active, so that thrombosis was not a cogent problem, also due to their relatively short lifespan. On the other hand, the modern man can become much older, is often overweight and physically inactive, so that the prothrombotic impact of the gain-of-function mutations is overtaking the protective effects on hemorrhage. The protection from thrombosis conferred by inherited disorders of hemostasis gave no evolutionary advantage to the primitive man, and affected individuals usually bled to death at birth or very early afterwards. The most likely explanation for the maintenance of hemophilia throughout the centuries is the high rate of spontaneous mutations in the corresponding genes.

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