8

Estrogens and hemostasis

Hemostasis is the physiological response to any vascular injury. Several mechanisms are concerned, involving platelets and proteins (factors of coagulation and fibrinolysis). Hemostasis is controlled by natural anticoagulants – antithrombin (AT), protein C (PC), protein S (PS) – and by the fibrinolytic system. An imbalance between the activation of hemostasis and these regulation mechanisms may lead to thrombosis.

The activation of hemostasis results in the generation of thrombin following a cascade of enzymatic reactions, involving both coagulation factors and platelets. Thrombin is a potent activator of platelets and coagulates fibrinogen. In the absence of vascular injury, its production is normally limited by the natural anticoagulant systems.

Antithrombin, a circulating protein activated by heparin and the heparan sulfates of the vascular wall, forms inactive complexes with thrombin and the other activated (a) factors of the coagulation system (F Xa, F IX a, etc.).

The protein C system is based upon the activation of PC by thrombin bound to thrombomodulin at the surface of endothelial cells. Activated PC (APC), associated with its cofactors PS, can then inactivate Factor Va and Factor VIIIa through proteolysis.

The importance of these inhibitors (AT, PC, PS) is shown by the occurrence of venous thromboembolic complications in patients suffering from hereditary thrombophilia (Aiach et al., 1995). Ten to fifteen per cent of subjects with a history of deep venous thrombosis (whatever have been the circumstances) present an hereditary deficiency in one of these proteins. The most frequent deficiency is APC resistance due to a mutation in one of the cleavage points of Factor V by APC - Arg 506 Gln or Leyden mutation of F V – (Dahlbäck, 1993 ; Bertina et al., 1994 ; Svensson and Dahlbäck, 1994). In European countries, this mutation affects 2 to 10 per cent of the general population and accounts for twenty to thirty per cent of deep venous thrombosis.

Fibrinolysis depends upon plasmin, the only enzyme capable of lysing the fibrin network of a thrombus. Plasmin production is linked to the activation of circulating plasminogen by t-PA and u-PA, factors released by the vascular wall. The action of these activators is restricted by the presence of plasminogen activator inhibitor (PAI) in the blood. The activity of the fibrinolytic

system is assessed by circulating d-dimers generated following fibrin degradation.

The risk of venous thrombosis is clearly associated with hypercoagulability and the main risk factors are coagulation inhibitor deficiencies (AT, PC, PS), as well as the Arg 506 Gln mutation in Factor V. Increases in Factor VIII and prothrombin (linked to a mutation of the untranslated 3' end of the gene) as well as blood group A have been associated with the venous thrombosis risk (Koster et al., 1995 ; Poort et al., 1996).

Paradoxically, the abnormalities described above do not result in a higher risk of arterial thrombosis (Emmerich et al., 1995). In contrast, epidemiological studies have shown clearly that fibrinogen, Factor VIII and fibrinolysis proteins (t-PA and PAII) are involved in the risk of acute ischemia in atheromatous patients and that the risk increases with the concentration of these proteins.

Thrombotic risk and estrogens

Estrogens modify the hemostasis balance and their use as oral contraceptives is clearly related to a higher risk of both venous and arterial thrombosis. It has recently been suggested that oral contraceptive users carrying the Arg 506 Gln mutation have a greater risk of venous thrombosis (Vandenbroucke et al., 1994).

Three studies have recently pointed out a potential risk of venous thrombosis (deep venous thrombosis and pulmonary embolism) in postmenopausal women treated with estrogens (Daly et al., 1996 ; Grodstein et al., 1996 ; Jick et al., 1996). It is not known whether subpopulations with genetic risk factors such as the Arg 506 Gln mutation might be more exposed to venous thrombosis on HRT.

It remains to be proved that the risk of arterial ischemic events (cardiovascular risk) is enhanced among postmenopausal women. A number of parameters are involved including not only the hemostasis system but also metabolic factors such as cholesterol and triglycerides. Several studies suggest that HRT could decrease the cardio-vascular risk through the influence of estrogens on lipids and hemostasis (for review, see Chae et al., 1997).

Influence of estrogens on hemostasis

Natural or synthetic estrogen administration may induce a number of modifications in the parameters of hemostasis (for review, see Meade, 1997). These modifications can impair the balance between prohemostatic factors and physiological antithrombotic mechanisms.

Tableau 8.I : Cellular receptors involved in hemostasis

Cell	Receptor	Role in hemostasis
Platelet	Integrin α2b-β3 (PG IIb/IIIa)	Platelet aggregation
Platelet	PG Ib/IX	Platelet adhesion
Platelet	Thrombin receptor	Aggregation and TXA2 synthesis
Endothelial cell	Thrombomodulin	Protein C activation
Endothelial cell and monocyte	Tissular factor (inducible)	Non-expressed in basal conditions Induced by inflammatory cytokines

The hormonal treatment of menopause induces modifications of various parameters which can be classified as follows :

- risk factors involved in thromboembolic venous disease : AT, PC, PS, F II, F V and F VIII.
- risk factors involved in ischemic cardiovascular events : fibrinogen, F VII, t-PA and PAI.The results of studies reported in the literature are summarized and discussed in the chapters 10 and 11. The modification of the different factors involved in the arterial as well as the venous risk depends on the type of treatment ; the route of administration (oral or transdermal) is essential (see chapter 11).
- modification of the balance between activators and inhibitors : fragments 1+2 of prothrombin (F1+2) and fibrinopeptides A (FPA) released after prothrombin and fibrinogen activation, respectively, or the presence of thrombin-antithrombin (TAT) complexes, are markers of coagulation activation ; an increase in such markers is an excellent marker of a change in the hemostasis balance, particulary in case of coagulation inhibitor deficiencies. Similarly, D-dimers formed after fibrin lysis can reflect stimulation of fibrinolytic activity, generally due to decreased PAI-1 levels (Koh et al., 1997).

Usually, whatever the regimen, HRT can reverse the fibrinogen increase observed following menopause (PEPI Trial Group, 1995). When other parameters, such as PAI-1, F VII and AT, are considered, the modifications elicited are not constant, presumably because of the heterogenity of the therapeutic protocols and the small number of patients in the studies.

Overall, the changes obtained, if confirmed, would be consistent with a reduced risk of arterial thrombosis, whereas they could result in an increased risk of venous thrombosis :

- the decrease in AT and PS could explain the risk of venous thrombosis.
- the decrease in PAI-1 and fibrinogen reversion to pre-menopausal levels could explain why the risk of arterial ischemic events is lowered.

It has to be underlined that the responses obtained are very different according to the type of treatment (chapter 11) and that, in one study, natural

estrogens administered transdermally appeared to be « neutral », suggesting that they do not increase the risk of venous thrombosis (Scarabin et al., 1997) but may be less efficient on the arterial risk.

Mechanism of action

Although the effect of estrogens on the circulating coagulation proteins have been known for years – particularly in the case of oral contraception – limited data are available on their mechanisms of action. Previous experimental studies performed on rats or dogs suggest that estrogens can interfere with the synthesis of AT (Koj et al., 1978 ; Kobayashi and Takeda, 1977). At the gene level, the only studies devoted to hormonal regulation concern F XII and F IX, whose promoters contain structures controlling respectively the response to estrogens (Farsetti et al., 1995) and to androgens (Crossley et al., 1992).

Cis-activating sequences, controlling the response to estrogens, have never been identified in the promoters of the genes coding for other hemostasis proteins. Thus, estrogens could work via mediators acting themselves at the promoter level.

Another hypothesis is direct activation of hemostasis – the mechanism of which would have to be identified – leading to utilization of coagulation inhibitors and thus a decrease in all of them. Since AT and PS decrease under estrogen therapy, whereas PC increases, this consumption mechanism is not likely to occur.

Data concerning the cellular mechanisms involved in hemostasis are also very few. The main membrane proteins contributing to this system are shown in table 8.I.

No studies devoted specifically to these receptors have been reported, but some effects of estrogens on endothelial cells, platelets and monocytes have been described (White et al., 1995 ; Ranganath et al., 1996). Estrogens could elicit a rise in the endothelial synthesis of prostacyclin (PGI2) and a decrease in the platelet synthesis of thromboxane A2 (TxA2) and in the expression of tissue factor by circulating monocytes (Durand and Blache, 1996). Such an effect would downregulate hemostasis.

To conclude, estrogens and progestogens modify the balance between hemostasis and the natural antithrombotic mechanisms, but the effect of such modifications on the risk of venous or arterial thrombosis remains to be established. The mechanism by which these hormones influence the expression of circulating proteins or cellular receptors involved in hemostasis also needs to be clarified.

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