APC resistance and third-generation oral contraceptives

Acquired resistance to activated protein C, oral contraceptives and the risk of thromboembolic disease

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Using a newly-developed technique, a severe acquired plasma resistance to activated protein C has been described in women using third-generation (rather than second-generation) oral contraceptives. The following items are discussed: (i) the technical parameters used to appreciate the effect of activated protein C induce a bias of interpretation, the mean intrinsic effect of activated protein C, in plasmas from women on second or third-generation oral contraceptives being strictly identical; (ii) there are no data available to show that this assay can indicate a thromboembolic risk in asymptomatic women on oral contraceptives; and (iii) this assay is a global and non-specific test, basically sensitive to the plasma concentrations of many coagulation factors which are increased or decreased by oestrogens and progestogens. For instance protein S, in which oral contraceptive-induced modifications account for the differential effect of oral contraceptives on Rosing’s assay, but which modifications are not related to the thromboembolic risk of oral contraceptives. The androgenic potential of the progestogen may counteract the effect of oestrogens in the test. More generally, in such a complex situation in which there is a ‘modification of the modification’, there is no haemostasis-related test which provides a risk indicator for thrombosis. Based on testing of the plasma response to activated protein C, it is impossible to state that third-generation oral contraceptives induce a more important thromboembolic risk than oral contraceptives containing a more androgenic progestogen.

Key words: acquired resistance/oral contraceptives/protein C/third-generation/thromboembolism

Introduction

Thromboembolism is a well-known complication of oral contraceptives (WHO Collaborative Study, 1995a). Oestrogens are considered to be responsible for the development of a hypercoagulable state that increases the risk for thromboembolic events, leading to the introduction of drugs containing a lower dose of oestrogen. In the 1980s, the introduction of third-generation drugs containing progestogens, e.g. desogestrel, gestodene and norgestimate, was reported to increase the risk of venous thromboembolism (WHO Collaborative Study, 1995b; Spitzer et al., 1996). Evaluations which were carried out on these initial data and on other studies attributed a significant part of this increased risk to properties of the users, and pointed to three main biases: prescription, referral, and healthy users.

The inherited deficiency of the anticoagulant response to activated protein C is a risk factor for venous thromboembolism. The congenital functional plasma phenotype termed ‘resistance to activated protein C’ was first described in 1993 (Dahlbäck et al., 1993). Inherited resistance to activated protein C is mainly caused by a single point mutation in factor V gene, leading to the synthesis of a molecular variant: factor V Leiden (Bertina et al., 1994). The thromboembolic risk of patients carrying this mutation is increased 30-fold by the use of oral contraceptives: in homozygotes, the risk is greater at >100-fold (Vandenbroucke et al., 1994).

Early reports have shown that, in non-carriers of the factor V Leiden mutation who are current users of oral contraceptives, the anticoagulant response to activated protein C is reduced (Osterud et al., 1994; Henkens et al., 1995; Olivieri et al., 1995). This led to the concept of acquired resistance to activated protein C (abnormal phenotype but normal genotype) being induced, in this case, by oral contraceptives. More generally, reduced sensitivity for activated protein C in the absence of factor V Leiden mutation has recently been described to increase the risk of venous thrombosis (DeVisser et al., 1999).

A new methodology for testing response to activated protein C has recently been described (Rosing et al., 1997). The authors showed that third-generation oral contraceptives induce a more important impairment of the plasma response to
activated protein C than do second-generation oral contraceptives.

The comparative appraisal of the side-effects of second and third-generation oral contraceptives is still a matter of debate. This led us to perform an in-depth re-evaluation of the links between oral contraceptives, the induced modifications of the plasma response to activated protein C and the thromboembolic risk, according to the available haemostasis-related tests used to characterize the plasma response to activated protein C.

Concerning the results of Dalbäck’s assay in women taking second or third-generation oral contraceptives, in the absence of factor V Leiden mutation

Dalbäck’s assay (Dalbäck et al., 1993) evaluates the increase of the activated partial thromboplastin time (aPTT) induced by a fixed amount of exogenous activated protein C: the less the aPTT increases, the more the plasma is resistant to the inhibitory action of activated protein C on coagulation factors V and VIII.

In the initial case-control study showing that an acquired plasma resistance to activated protein C is associated with an increased thromboembolic risk (DeVisser et al., 1999), women had lower values than men, an increase in age was associated with a decrease in values and high factor VIII plasma concentrations were associated with low responses. After adjustment for sex, age and factor VIII concentrations, low values still predicted a 2.5 excess of clinical risk. Women not on oral contraceptives had slightly (but not significantly) higher values than the group of women as a whole. This let readers suspect that women on oral contraceptives had not significantly lower values than the group of women as a whole: however, specific data concerning women on oral contraceptives were not shown and no data concerning the difference between second and third-generation compounds users were given.

The same team had previously shown that thrombosis patients using oral contraceptives have lower responses to activated protein C than thrombosis patients not using oral contraceptives, and that thrombosis patients using oral contraceptives have lower results than non-thrombosis controls using oral contraceptives (Bloemenkamp et al., 1998). The percentage of ‘high responders’, i.e. those below the 25th percentile of the plasma response to activated protein C evaluated in controls in former thrombosis patients and in healthy control women, both using oral contraceptives, was 50 and 25% respectively (Bloemenkamp et al., 1998). This favours the hypothesis that the oral contraceptive-induced variations of response to activated protein C are more important in former thrombosis patients than in asymptomatic women (Bloemenkamp et al., 1998) and shows that an acquired resistance to activated protein C is likely to be relevant in the pathogenesis of venous thrombosis induced by oral contraceptives. However, no data are available on the differential effects of second and third-generation drugs.

In a study comparing the performances of two different functional tests, no significant difference in sensitivity to activated protein C between users of second (n = 62) or third-generation (n = 64) oral contraceptives was observed using Dalbäck’s assay (Curvers et al., 1999). More recently, a cycle-controlled cross-over study performed on 28 women who were not using oral contraceptives showed initially a more pronounced resistance to activated protein C induced by a 150 µg desogestrel-containing oral contraceptives than by a 150 µg levonorgestrel-containing oral contraceptive, both of them containing 30 µg ethinyl oestradiol (Tans et al., 2000).

Thus, using Dalbäck’s assay, an acquired low response to activated protein C is more likely to be associated with venous thrombosis in women on oral contraceptives. In normal asymptomatic women, a desogestrel-containing third-generation oral contraceptive induces a more important impairment of the plasma response to activated protein C than does the corresponding levogestrel-containing oral contraceptive. However, there is no evidence to show that this induced modification of plasma response to activated protein C is related to the clinical thromboembolic risk.

Concerning the results of Rosing’s assay in women taking second or third-generation oral contraceptives, in the absence of factor V Leiden mutation

Thrombin, the coagulation terminal enzyme, plays a key role in the genesis of venous thromboembolic disease. The assay developed by Rosing et al. (1997) in order to quantify the response to activated protein C is based on the quantification of the endogenous thrombin potential (ETP) previously described by Hemker and Begin (1995), which basically evaluates the plasma concentration of free-generated thrombin, as a function of time, in a plasma milieu in which coagulation is activated (Rosing et al., 1997; Table I). In this assay, coagulation is activated by low-dose tissue factor, thus through activated factor VII, and not by contact phase activation as it is performed in the aPTT-based Dalbäck’s assay. The ETP is defined as the time-integral of free thrombin concentration. The thrombin generation curves are performed in the absence (basal ETP) then in the presence (ETP-aPC) of a fixed amount of activated protein C (aPC), the physiological coagulation inhibitor, and the total amounts of thrombin generated in the two situations are then compared. The ETP-aPC value is lower than the basal ETP value, due to the inhibitory action of aPC. In order to establish a relative appraisal of the effect of aPC, the ETP-aPC value was first proposed to be evaluated as a percentage of the basal ETP, then as the aPC sensitivity ratio (aPC-sr) (Rosing et al., 1997; see Table I).

Some of the data published in Rosing’s paper are given in Table II. Accordingly, oral contraceptives induce an acquired resistance to activated protein C. Third-generation components have a more deleterious effect than second-generation ones, with mean ETP-aPC values very close to the one observed in the case of heterozygous factor V Leiden mutation. Careful observation of the data leads to the detection of higher values of basal ETP in plasmas of women taking triphasic oral contraceptives and third-generation oral contraceptives: in such a situation, the quantification of the effect of aPC on ETP using the expression of ETP-aPC values as a percentage of the basal EPT values induces a perception and interpretation bias. When the difference between basal ETP and ETP-aPC
is calculated in plasmas from women taking second or third-generation oral contraceptives, activated protein C induces the same absolute effect (Table II). The two generations of oral contraceptives cannot thus be separated on the basis of the intensity of their induced acquired resistance to activated protein C.

The plasma response to activated protein C is thereafter given as the aPC sensitivity ratio (aPC-sr), which is introduced as a marker of plasma resistance to activated protein C. Basically, the aPC-sr is high when the studied plasma has a high ETP-aPC/basal PTE ratio. This can be the consequence of a high ETP-aPC value or/and of a low basal PTE value. In Rosing’s paper, the use of the aPC-sr is associated with higher values in women on third-generation oral contraceptives than in women on second generation ones, despite a similar absolute plasma effect of activated protein C (Table III). Under these circumstances, the use of the aPC-sr is no more convincing: it is very easy to demonstrate, on a mathematical basis, that the association of a higher basal ETP with an unchanged intrinsic effect of aPC on thrombin generation always generates an increased aPC-sr value. The aPC-sr parameter, due to its structure, amplifies the bias.

Similarly, the recent report of the results of a cross-over study showing that a desogestrel-containing oral contraceptive induces a more important impairment of the aPC-sr than does a levonorgestrel-containing oral contraceptive (Tans et al., 2000) is no more convincing. The values of the basal ETP obtained during the two treatments are not given.

The significance of higher basal ETP values in women on third-generation or triphasic oral contraceptives must be discussed. A basal ETP performed according to Rosing’s technique has never been, in case-control or prospective studies, associated with an increased thrombotic risk. Among pregnant women who either do or do not carry the heterozygous factor V Leiden mutation, the basal ETP performed at 12, 22 and 34 weeks of amenorrhoea, or 3 months after delivery, do not significantly vary and show similar values in carriers and non-carriers of the mutation (Eichinger et al., 1999). In such circumstances, markers of haemostasis activation increase. The factor V Leiden gene mutation, however, is known as a real

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**Table I.** New method used to quantify the plasma response to activated protein C (Rosing et al., 1997)

<table>
<thead>
<tr>
<th>Determination of the basal endogenous thrombin potential (ETP)</th>
</tr>
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<tbody>
<tr>
<td>Use of defibrinated plasma</td>
</tr>
<tr>
<td>Induction of coagulation by a fixed low dose of relipidated purified tissue factor, in presence of calcium</td>
</tr>
<tr>
<td>Regular quantification of the free thrombin concentration in the plasma milieu (specific amidolytic activity)</td>
</tr>
<tr>
<td>Construction of the thrombin generation curve as a function of time</td>
</tr>
<tr>
<td>Calculation of the ETP, defined as the time-integral of free thrombin concentration detected in the test</td>
</tr>
<tr>
<td>Using another volume of the same plasma sample, determination of the ETP in presence of a fixed quantity of purified exogenous activated protein C (ETP-aPC)</td>
</tr>
<tr>
<td>Activated protein C, together with protein S, inactivates endogenous activated factor V and, together with factor V, endogenous activated factor VIII</td>
</tr>
<tr>
<td>The coagulation system thus lacks its two active coenzymes</td>
</tr>
<tr>
<td>Despite a similar coagulation activation, the thrombin generation curve is thus impaired</td>
</tr>
</tbody>
</table>

**Exploitation of the obtained results**

First proposal: the ETP-aPC is given as a percentage of the basal ETP (%)

Second proposal: calculation of the aPC sensitivity ratio (aPC-sr) concerning patient’s plasma sample ‘P’:

- use of the basal ETP and of the ETP-aPC values; calculation of the ratio [ETP-aPC/basal ETP] = ratio ‘P’ using normal plasma pool ‘N’;
- determination of the basal ETP and of the ETP-aPC values; calculation of the ratio [ETP-aPC/basal ETP] = ratio ‘N’; finally, aPC-sr ‘P’ = [ratio ‘P’/ratio ‘N’]

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**Table II.** Effect of activated protein C (aPC) on the endogenous thrombin potential value (ETP: nm min), according to published data (Rosing et al., 1997). Values are given as means with percentage shown in parentheses. The last column of the table is calculated from the data given in the referenced paper.

<table>
<thead>
<tr>
<th>Origins of pooled plasmas</th>
<th>basal ETP</th>
<th>ETP-aPC</th>
<th>basal ETP – ETP-aPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasmas (n = 90)</td>
<td>458</td>
<td>43 (9.4)</td>
<td>415</td>
</tr>
<tr>
<td>Men (n = 23)</td>
<td>442</td>
<td>39 (8.8)</td>
<td>403</td>
</tr>
<tr>
<td>Women</td>
<td>473</td>
<td>45 (09.5)</td>
<td>428</td>
</tr>
<tr>
<td>No oral contraceptive (n = 27)</td>
<td>533</td>
<td>105 (19.7)</td>
<td>428</td>
</tr>
<tr>
<td>Triphasic oral contraceptive (n = 28)</td>
<td>483</td>
<td>85 (17.6)</td>
<td>398</td>
</tr>
<tr>
<td>Monophasic oral contraceptive</td>
<td>554</td>
<td>155 (28.0)</td>
<td>399</td>
</tr>
<tr>
<td>Third generation (n = 25)</td>
<td>486</td>
<td>141 (29.0)</td>
<td>345</td>
</tr>
</tbody>
</table>

OC = oral contraceptives; ETP-aPC = ETP performed in presence of exogenous purified aPC.
Table III. Effect of a high basal endogenous thrombin potential (ETP) associated with a constant intrinsic effect of activated protein C (aPC) on the aPC sensitivity ratio value (aPC-sr). Values of basal ETP and of ETP-aPC correspond to the published mean values (Rosing et al., 1997)

<table>
<thead>
<tr>
<th>Values of the obtained activities:</th>
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<tbody>
<tr>
<td>Normal plasmas:</td>
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<tr>
<td>Basal ETP = 458, ETP-aPC = 43</td>
</tr>
<tr>
<td>Absolute difference: 415</td>
</tr>
<tr>
<td>Plasmas of women on second generation oral contraceptives:</td>
</tr>
<tr>
<td>Basal ETP = 483, ETP-aPC = 85</td>
</tr>
<tr>
<td>Absolute difference: 398</td>
</tr>
<tr>
<td>Plasmas of women on third generation oral contraceptives:</td>
</tr>
<tr>
<td>Basal ETP = 554, ETP-aPC = 155</td>
</tr>
<tr>
<td>Absolute difference: 399</td>
</tr>
</tbody>
</table>

Corresponding calculated values of the aPC-sr:
Normal plasmas: 1
Plasmas of women on second generation oral contraceptives:
[085/483]/[43/458] = 0.17599/0.0939 = 1874
Plasmas of women on third generation oral contraceptives:
[155/554]/[43/458] = 0.2798/0.0939 = 2979

risk factor of thrombosis during pregnancy (Grandone et al., 1998). Thus, the clinical relevance of Rosing’s basal ETP deserves to be demonstrated.

A more fundamental criticism applies to the aPC-sr: there is no available clinical study showing that this parameter can indicate a thromboembolic risk. A case-control study nested in a vast German cohort studied the thromboembolic risk associated with abnormal aPC-sr values, using Rosing’s test (Heinemann et al., 1998). In the absence of the factor V gene Leiden mutation, the aPC-sr does not indicate a clinical risk. Thus, acquired abnormal values of the aPC-sr do not have any clinical value.

Finally, using Rosing’s test, women on third-generation oral contraceptives do not have any particularly abnormal acquired failure of the plasma anticoagulant activity induced by exogenous activated protein C. Third-generation oral contraceptives induce complex modifications of Rosing’s test which do not depend on the absolute effect of activated protein C. Until now, these modifications have not been successfully related to the venous thromboembolic risk.

Other questions concerning the response to activated protein C in women taking second or third-generation oral contraceptives, in the absence of factor V Leiden mutation

Unfortunately, the effect of activated protein C has not been studied using a more physiological and global approach. The protein C–protein S–factor V system is activated when thrombin binds to its physiological endothelial receptor, thrombomodulin, and activates protein C. Protein C is bound to the endothelial surface by the protein C receptor. Thus, the endogenous thrombin generated during coagulation activation also acts as a physiological anticoagulant through protein C activation if thrombomodulin and protein C receptor are available. That is reminiscent of the description of the original method in which an ETP determination was performed using a plasma-containing purified thrombomodulin (Duchemin et al., 1994). Sophisticated methods in which coagulation activation may be evaluated in cellular systems containing platelets, monocytes and endothelial cells may help to clarify the differential effect, if any, of second and third-generation oral contraceptives.

The aPC-sr parameter has been used to demonstrate the importance of the dose of levonorgestrel, a progestogen which is contained in some monophasic second-generation and triphasic oral contraceptives (Kluft et al., 1999). This work shows a negative effect of the mean daily dose of levonorgestrel on the aPC-sr parameter, the highest doses counteracting the increase of the aPC-sr induced by oral contraceptive intake. Thus, levonorgestrel induces plasma modifications able to modulate, in vitro, the results of a clinically non-informative biological test.

A recent cycle-controlled randomized cross-over trial aimed to compare the effect of a second-generation (progestogen: 150 µg levonorgestrel) with that of a third-generation (progestogen: 150 µg desogestrel) oral contraceptive, each of them containing 30 µg ethinyl oestradiol (Rosing et al., 1999). This elaborate study shows that the aPC-sr parameter increases with both compounds, with a higher increase in women on third-generation contraceptives. Increases of factor II, factor VII and thrombin-activated fibrinolysis inhibitor (TAFI), together with decreases of factor V and protein S were also shown. Very good positive linear correlations between basal and on treatment aPC-sr values have been demonstrated for both progestogens, with a higher slope for desogestrel (on treatment mean aPC-sr value of 4.5 for a basal value of 3.0) than for levonorgestrel (3.5 for 3.0). This indicates that the basal aPC-sr value depends on plasma parameters which are modified, with a mean intensity which depends on the type of progestogen, by the type of oral contraceptive. Thus, the basal aPC-sr value incorporates the value of the aPC-sr on oral contraceptives. The main criticism is that there is no available evidence that oral contraceptive-induced modifications of any biological parameter can be clinical risk indicators when their basal values (if any), have a documented status as an epidemiological indicator. A typical example is protein S, a co-factor of the protein C anticoagulant system. Its plasma concentrations decrease on oral contraceptives: studies have failed to demonstrate that it indicates a risk of thrombosis. We suspect that the variations of aPC-sr resulting from oral contraceptives merely reflect the numerous variations of the haemostasis factors induced by oestrogens and progestogens. Correlations between values of aPC-sr obtained before and while on oral contraceptives probably only reflect the complex positive and negative variations of coagulation factors induced by the various hormonal compounds. This has been confirmed in a recent cross-over study published by Rosing et al. (1999) demonstrating that the ETP-based assay is influenced by protein S plasma concentrations, particularly of free protein S, which are decreased by desogestrel but not by levonorgestrel (Tans et al., 2000). Thus, so far, the impairment of the ETP-based aPC-sr observed with third-generation oral contraceptives and modulated by changes in protein S, have not yet been.
related to the thromboembolic risk. This discrepancy has recently been elegantly summarized (Kluft, 2000): a drug may influence a global or non-specific test, sensitive to factors A to D, via a factor B that can show variations without clinical consequences. However, the variations in the test may also be associated with a clinical endpoint via its sensitivity to variations in factor D, when variations in factor D concentrations are clinically relevant. Thus, due to the factor which is influenced by a given drug, the same absolute variations of a non-specific test may be clinically relevant or not. Our goal is now to find the clinically-relevant factor D!

Finally, the aPC-sr parameter cannot yet be validated as an indicator of risk in asymptomatic women. A recent communication (Tans et al., 1999) of a work performed using the plasmas of the Leiden Thrombophilia Study has shown the existence of a higher venous thrombotic risk predicted by the aPC-sr parameter, but only in symptomatic men with a primary thrombotic accident. Moreover, this risk only appears in values of the aPC-sr parameter of the higher quartile compared to values of the lower quartile, the lower limit of the 95% confidence interval of the odds ratio being 1.1, i.e. close to 1. We have no data on asymptomatic women on oral contraceptives.

Oestrogens and androgens often have opposite hepatic effects, leading to opposite mean modifications of the plasma concentrations of various proteins: for instance, the sexual steroids binding protein, antithrombin, triglycerides, high density lipoprotein (HDL) cholesterol, etc. There is no evidence available to show that modifications of plasma concentrations induced by oestrogens have the same epidemiological significance as do basal concentrations. For instance, we currently do not know if the increase in HDL cholesterol induced by an oral oestrogen has the same protector effect against coronary diseases as does the basal HDL cholesterol concentration.

Neither is there any available demonstration to show that the addition of the opposite effects, on plasma concentrations of an indicator of clinical risk, of an oestrogen and a progestogen leads to cancellation of the clinical risk associated with the modifications of the given biological indicator of risk induced by the oestrogen or progestogen alone. For instance, taking both an androgen and an oestrogen together leads to a decrease in the plasma HDL cholesterol concentrations, but we currently cannot affirm that it modifies the effect of oral oestrogens alone on atherogenesis, if any. It seems obvious, but not demonstrated, that oestrogens alone modify the basal value of the aPC-sr parameter, with no demonstration of a link between this modification and the induced thromboembolic risk. Taking an androgenic progestogen with oestrogens, probably lowers the induced modification of the aPC-sr (as for the other hepatic effects of oestrogens), without any demonstration that this ultimate modification, the ‘modification of q modification’, lowers the risk of a hypothetical clinical consequence. The recorded dose effect of levonorgestrel back up this interpretation. Third-generation progestogens, which have fewer androgenic properties, probably have a lower attenuation effect than second-generation progestogens on the basal aPC-sr value. Thus, it would seem very hazardous to claim that it induces, de facto, a more important thromboembolic risk than when an androgenic progestogen is used.

Conclusions

Analysis of the biological elements recently used to argue that there is a higher thromboembolic risk in women on third-generation rather on second-generation oral contraceptives, namely by induction of a more severe acquired plasma resistance to activated protein C, does not support this conclusion.

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References


