Influence of oral contraceptive use on endothelial t-PA release in healthy premenopausal women

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Hoetzer, Greta L., Brian L. Stauffer, Jared J. Greiner, Yoli Casas, Derek T. Smith, and Christopher A. DeSouza. Influence of oral contraceptive use on endothelial t-PA release in healthy premenopausal women. Am J Physiol Endocrinol Metab 284: E90–E95, 2003. First published August 20, 2002; 10.1152/ajpendo.00333.2002.—We determined the influence of oral contraceptives (OC) on the capacity of the endothelium to release tissue-type plasminogen activator (t-PA). Twenty-three healthy premenopausal women were studied: 12 nonusers and 11 users of OC. Net endothelial release rates of t-PA were calculated as the product of the arteriovenous concentration gradient and forearm plasma flow in response to intra-arterial bradykinin (BK: 12.5–50 ng·100 ml tissue−1·min−1) and sodium nitroprusside (SNP: 1.0–4.0 μg·100 ml tissue−1·min−1). Net release of t-PA antigen and increment in t-PA activity across the forearm to BK increased (P < 0.01) in a dose-dependent fashion and to similar extents in the nonusers and users of OC. At the highest BK dose, net release of t-PA antigen was 64.5 ± 8.2 and 66.2 ± 15.4 ng·100 ml tissue−1·min−1 in the nonusers and users of OC, whereas the net increment in t-PA activity was 18.6 ± 3.0 and 16.0 ± 2.0 IU·100 ml tissue−1·min−1, respectively. There was no effect of SNP on t-PA release in either group. These results indicate that endothelial t-PA release is not altered in premenopausal women who use oral contraception.

fibrinolysis; endothelium; tissue-type plasminogen activator; bradykinin; contraceptives

It is estimated that more than 100 million women worldwide use oral contraception (42). Clinical and epidemiological data indicate that oral contraceptive use is associated with an increased risk of thrombotic disorders, including myocardial infarction, deep vein thrombosis, and venous thromboembolism (16, 39, 40, 41). In fact, the risk of thrombosis has been reported to be two- to fourfold greater in users compared with nonusers of oral contraceptives, independent of smoking status (15, 41). The mechanisms underlying this apparent prothrombotic state are not clear. Considerable attention has focused on the coagulation-fibrinolysis axis, specifically, whether the procoagulant effects of oral contraceptives are balanced by increased fibrinolytic capacity.

Several studies have reported that oral contraceptives lower circulating plasma concentrations of tissue-type plasminogen activator (t-PA) antigen, plasminogen activator inhibitor-1 (PAI-1) antigen and activity, and increase levels of t-PA activity, suggesting that fibrinolytic activity is increased with oral contraceptive use (14, 20, 29). However, favorable alterations in circulating levels of t-PA and PAI-1 induced by oral contraceptive use may not necessarily reflect increased fibrinolytic activity per se but rather changes in hepatic clearance (20). Moreover, recent data indicate that it is the capacity of the endothelium to release t-PA rapidly and acutely from intracellular storage pools and not circulating plasma fibrinolytic concentrations that determines the efficacy of endogenous fibrinolysis (13, 35, 40a). Indeed, thrombolysis is more effective if active t-PA is readily available during, rather than after, thrombus formation (1, 9). Furthermore, the inhibitory interaction between PAI-1 and t-PA has a second-order rate constant of ~107 M−1·s−1 (33); therefore, local rapid release of t-PA is critical to the fibrinolytic process. Although the effects of oral contraceptives on plasma markers of fibrinolysis have been well studied, little information is available regarding the impact of oral contraceptives on endothelial t-PA release.

Accordingly, the aim of the present investigation was to determine the influence of oral contraceptive use on the capacity of the vascular endothelium to release t-PA in healthy premenopausal women. To address this aim, we employed an isolated forearm model to determine, in vivo, rates of endothelial t-PA release in well-matched healthy premenopausal women who were either taking or not taking oral contraceptives.

MATERIALS AND METHODS

Subjects. Twenty-three healthy sedentary premenopausal women were studied: 12 nonusers (age 21–40 yr) and 11 users of oral contraceptives (22–38 yr). All women were

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nonobese, normotensive (blood pressure <140/90), nonsmokers, eumenorrheic, and free of overt disease, as assessed by medical history and fasting blood chemistries. Women not using oral contraceptives had discontinued use for at least 1 yr before the start of the study. The women taking oral contraceptives had done so continuously for at least 6 mo (range: 0.5–12 yr) before the study (Table 1). Five of the 11 women using oral contraception were taking second-generation and six were taking third-generation oral contraceptives. All women were studied during the follicular phase of their menstrual cycle. In addition, all subjects were free of recent infection/inflammation (<2 wk) as determined by their menstrual cycle. Drug infusion rates were normalized per 100 ml forearm tissue and infused at 4 ml/min by a syringe pump. After the measurement of resting blood flow for 5 min, bradykinin was infused intra-arterially at 12.5, 25, and 50 ng·100 ml tissue⁻¹·min⁻¹ and sodium nitroprusside at 1.0, 2.0, and 4.0 μg·100 ml tissue⁻¹·min⁻¹ for 5 min at each dose. To avoid an order effect, the sequence of drug administration was randomized.

Blood sampling and biochemical assays. All phlebotomies were performed with minimal venostasis. Arterial and venous blood samples were collected simultaneously at baseline and the end of each drug dose to determine t-PA and PAI-1 antigen and activity concentrations. The first 3 ml of blood from both the artery and vein were discarded before each sample was collected. Blood samples were collected in tubes containing 0.45 M sodium citrate buffer, pH 4.3 (final dilution volume 1:10; Stabilyte; Biopool). Within 15 min of collection, all samples were centrifuged for 20 min at 5,000 g at 4°C. Platelet-poor plasma was separated into aliquots and stored at −70°C until assayed at the end of the study. All assays were performed in duplicate with a maximum of one freeze-thaw cycle. Plasma concentrations of t-PA and PAI-1 antigen and activity as well as factor VII-antigen (Ag) were determined by enzyme immunoassay (Biopool International, Ventura, CA, and Diagnostic, Stago, France). t-PA activity is expressed in international units, and PAI-1 activity is expressed in arbitrary units.

Net release of fibrinolytic factors across the forearm. Net endothelial release of t-PA and PAI-1 antigen and increase in t-PA and PAI-1 activity in response to bradykinin and sodium nitroprusside was calculated according to Jern et al. (13). Briefly, arteriovenous concentration gradients were determined by subtracting the measured values in simultaneously collected venous and arterial blood. For both t-PA and PAI-1, a positive difference indicated a net release, and a negative difference indicated net uptake. Net release or uptake rates were calculated as follows: net release = (C_v – C_a) × [FBF × (101 – hematocrit/100)], where C_v and C_a represent the concentration in the vein and artery, respectively. Hematocrit was measured in triplicate using the standard microhematocrit technique and corrected for trapped plasma volume within the trapped erythrocytes (3). The total amount of t-PA antigen released and total increment in t-PA activity across the forearm in response to bradykinin were calculated as the total area under each curve above baseline using a trapezoidal model.

Statistical analysis. Differences in subject baseline characteristics and area under the curve were determined by ANOVA. Differences between groups in net release of fibrinolytic factors across the forearm in response to incremental intra-arterial doses of bradykinin and sodium nitroprusside were determined by repeated-measures ANOVA. There were no significant differences in any of the key outcome variables between users of second- and third-generation oral contraceptives. Therefore, the data were pooled and presented together. All data are reported as means ± SE. Statistical significance was set at a priori at P < 0.05 for all comparisons.

RESULTS

Subject characteristics. Table 1 presents selected subject characteristics. There were no significant differences in anthropometric, hemodynamic, or meta-
Table 2. Circulating plasma concentrations of fibrinolytic and coagulation factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonusers of Oral Contraceptives</th>
<th>Users of Oral Contraceptives</th>
</tr>
</thead>
<tbody>
<tr>
<td>t-PA antigen, ng/ml</td>
<td>$6.4 \pm 0.6$</td>
<td>$3.3 \pm 0.8^*$</td>
</tr>
<tr>
<td>t-PA activity, IU/ml</td>
<td>$0.64 \pm 0.16$</td>
<td>$0.66 \pm 0.08$</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/ml</td>
<td>$9.1 \pm 2.2$</td>
<td>$2.8 \pm 0.6^*$</td>
</tr>
<tr>
<td>PAI-1 activity, AU/ml</td>
<td>$5.8 \pm 2.0$</td>
<td>$0.7 \pm 1.4^*$</td>
</tr>
<tr>
<td>Factor VII-Ag, %</td>
<td>$83 \pm 3$</td>
<td>$95 \pm 4^*$</td>
</tr>
</tbody>
</table>

Values means ± SE. t-PA, tissue-type plasminogen activator; PAI-1, plasminogen activator inhibitor-1; Ag, antigen. $^*P < 0.05$ vs. nonusers of oral contraception.

Vasodilator and fibrinolytic responses to bradykinin and sodium nitroprusside. As shown in Fig. 1, the FBF responses to bradykinin and sodium nitroprusside were almost identical between the groups. There were no significant group differences in basal endothelial release of t-PA antigen ($1.6 \pm 1.1$ vs. $0.4 \pm 0.9$ ng·100 ml tissue$^{-1}$·min$^{-1}$, $P = 0.44$) or increment in t-PA activity ($0.4 \pm 0.2$ vs. $0.4 \pm 0.1$ IU·100 ml tissue$^{-1}$·min$^{-1}$, $P = 0.71$) between the women taking and not taking oral contraceptives. In response to bradykinin, the net release of t-PA antigen and increment in t-PA activity increased in a dose-dependent fashion and to similar extents in the users and nonusers of oral contraceptives. At the highest bradykinin dose (50 ng·100 ml tissue$^{-1}$·min$^{-1}$), net endothelial release of t-PA antigen was $66.2 \pm 15.4$ ng·100 ml tissue$^{-1}$·min$^{-1}$ in the users compared with $64.5 \pm 8.2$ ng·100 ml tissue$^{-1}$·min$^{-1}$ in the nonusers (Fig. 2), whereas the peak increment in t-PA activity was $16.4 \pm 1.7$ and $18.6 \pm 3.0$ IU·100 ml tissue$^{-1}$·min$^{-1}$, respectively (Fig. 3). In addition, there were no group differences in either the total amount of t-PA antigen released (area under the curve: $299 \pm 72$ vs. $327 \pm 40$).
The primary new finding of the present investigation is that endothelial regulation of fibrinolysis appears to be unaffected by oral contraceptive use. We observed similar rates of basal and stimulated endothelial release of t-PA antigen and increment in t-PA activity across the forearm in healthy premenopausal women taking and not taking oral contraceptives. In addition, although endothelial t-PA release was not different between the groups, the women taking oral contraceptives demonstrated higher circulating levels of coagulation factor VII-Ag. It has been suggested that the procoagulant effects of oral contraceptives are counterbalanced by increased fibrinolytic potential (5, 28). The results of the present study question this postulate.

Premenopausal women who take oral contraceptives are at an increased risk of arterial and venous thrombosis (38–41). Although the precise mechanisms for the enhanced thrombotic risk associated with oral contraceptive use have not been completely elucidated, inadequate fibrinolysis as a causal factor has generally been dismissed. In fact, a number of studies have shown that oral contraceptive use is associated with favorable changes in plasma fibrinolytic markers suggestive of enhanced fibrinolytic activity (14, 20, 29). Our finding of lower plasma concentrations of t-PA antigen, PAI-1 antigen, and PAI-1 activity in the users compared with nonusers of oral contraceptives is in line with these previous reports. However, changes in steady-state plasma fibrinolytic concentrations provide an indirect and, in some cases, misleading assessment of endothelial t-PA release and, in turn, endogenous fibrinolytic potential (12, 13). Oral contraceptive-induced reductions in plasma t-PA and PAI-1 concentrations may reflect estrogen-associated changes in hepatic synthesis and clearance and not enhanced fibrinolysis (20). For example, estrogen has been reported to increase t-PA clearance through upregulation of the manose receptor, a hepatic clearance receptor for t-PA (23). In addition, the ineffectiveness of transdermal compared with oral estrogen administration in lowering basal PAI-1 levels in postmenopausal women supports the concept that the hepatic effects of estrogen are important in the regulation of PAI-1 synthesis and clearance (10, 22).

The salient and novel finding of the present study is that the capacity of the endothelium to acutely release t-PA is unaffected by oral contraceptive use. The magnitude of increase in net endothelial release of t-PA antigen and increment in t-PA activity across the forearm in response to bradykinin was not different between the women taking and not taking oral contraceptives and, importantly, was similar to levels previously reported in healthy young adults (2). Moreover, we observed no significant changes in t-PA release in response to sodium nitroprusside, indicating that the increases observed with bradykinin were not a blood flow-related phenomenon. Our in vivo findings are consistent with in vitro studies demonstrating no effect of estradiol, ethinyl estradiol, levonorgestrel, or gestodene on endothelial t-PA production (21). Considering t-PA is the key enzyme in initiating an endogenous fibrinolytic response, absence of greater endothelial t-PA release in premenopausal women taking oral contraceptives argues against the notion of increased fibrinolytic activation with oral contraceptive use.

Increased coagulation is thought to be a primary factor in the increased thrombotic risk associated with
oral contraceptives. Plasma concentrations of several markers of coagulation, including factor VII, VIII, and X and fibrinogen, have been shown to be higher and activity of the protein C pathway lower in users compared with nonusers of oral contraceptives (20, 26, 30). Our finding of significantly higher levels of factor VII-Ag in users compared with nonusers of oral contraceptives is in accordance with these previous reports. Importantly, however, despite evidence of increased coagulability, we observed no compensatory increase in endothelial t-PA release associated with oral contraceptive use in the present study. This finding is in line with that of Meijers et al. (29) who reported no effect of oral contraceptives on overall clot-lysis time, despite favorable changes in plasma fibrinolytic factors suggesting enhanced fibrinolytic activity. Collectively, the results of the present study and Meijers et al. (29) suggest that the procoagulant effects of oral contraceptives are not neutralized by enhanced fibrinolytic capacity. An imbalance between coagulation and fibrinolysis may indeed underlie the increased thrombotic risk associated with oral contraceptives.

It is noteworthy that none of the premenopausal women in the present study were smokers. Cigarette smoking has been shown to profoundly impair endothelial t-PA release throughout the vasculature (31, 32, 36). For example, Newby and colleagues (31, 32) reported that current and ex-smokers release significantly less t-PA in the forearm and coronary circulation compared with nonsmokers. In addition, reduced coronary t-PA release was associated with increased atheromatous plaque burden (31). This is particularly relevant given the fact that premenopausal women who smoke and take oral contraceptives are at 10–30 times greater risk of thrombotic events compared with their nonsmoking counterparts (37, 41, 43). It is likely that if smokers were included in the present study our results would have been different.

In addition to fibrinolytic function, the vascular endothelium plays an important role in the regulation of vascular tone through the synthesis and release of various vasodilating and vasoconstricting substances (24). One of the most important vasodilating substances released by the endothelium is nitric oxide (NO). Impaired NO-mediated endothelium-dependent vasodilation is thought to contribute to the etiology of atherosclerosis and thrombosis (4, 25). In the present study, the FBF responses to the endothelial agonist bradykinin were not significantly different between the users and nonusers of oral contraceptives. Our results compliment those of John et al. (17) who reported no impairment in ACh-mediated endothelium-dependent vasodilation in a similar group of healthy premenopausal women taking oral contraceptives. Both bradykinin and ACh are NO-dependent endothelial agonists; however, they stimulate endothelium-dependent vasodilation via different receptor-mediated mechanisms (34). Thus, taken together, these findings suggest that the vascular risk associated with oral contraceptive use does not appear to involve impaired endothelial vasodilator function.

Three experimental limitations of the present study should be mentioned. First, with all cross-sectional studies, it is possible that genetic and/or other lifestyle behaviors influenced the results of our group comparisons. We attempted to minimize potential lifestyle influences by studying healthy women across the premenopausal age range who were nonsmokers and did not differ in body composition or habitual physical activity. Second, potential regional differences in fibrinolytic activity limit extrapolation of our findings in the forearm to other vascular beds (11, 18). However, there is evidence to suggest that forearm endothelial t-PA release may be an excellent surrogate measure of t-PA release in the coronary vasculature (31, 32). Nevertheless, the results of the present study should be viewed within the context of the vascular bed studied. Finally, differences in thrombotic risk have been reported between oral contraceptives containing second-generation progestagens (primarily levonorgestrel) and third-generation progestagens (gestodene and desogestrel; see Refs. 38 and 39). A recent meta-analysis reported a 1.7-fold increased risk of venous thrombosis in women using third-generation compared with second-generation oral contraceptives (19). The mechanisms responsible for this increased risk are not clear. Although we observed no difference in t-PA release between the users of second- and third-generation oral contraceptives (total t-PA antigen released: 256 ± 76 vs. 333 ± 122 ng/min, P = 0.62), in the present study the small sample size, associated variability, and lack of statistical power limit interpretation. Future studies are needed to determine the independent effects of second- and third-generation oral contraceptives on endothelial t-PA release.

In conclusion, the results of the present study indicate that oral contraceptive use does not influence, either positively or negatively, endothelial t-PA release in healthy premenopausal women. The lack of a compensatory increase in endogenous endothelial fibrinolytic capacity to counteract enhanced coagulability may contribute mechanistically to the increased thrombotic risk associated with oral contraceptive use.

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