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# LONG-TERM TREATMENT WITH COMBINED ORAL CONTRACEPTIVES AND CIGARETTE SMOKING ASSOCIATED WITH IMPAIRED ACTIVITY OF TISSUE PLASMINOGEN ACTIVATOR

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*Abstract.* The fibrinolytic activity in vein walls (FAV) was determined by a semi-quantitative fibrin slide technique in a group of 68 healthy middle-aged women consisting of 22 healthy controls, 19 non-smoking contraceptive pill users and 27 cigarette-smoking non-users. Significantly decreased FAV was demonstrated in pill users because of high frequency of low values among women using combined oral contraceptives (OC) for more than 5 years. Significantly lower FAV was also recorded in smoking non-users, among whom low FAV values were found mainly in heavy smokers. However, no effect of OC usage for less than 5 years was observed, neither did smoking of less than 10 cigarettes daily influence FAV. Thus, the two well-known risk groups with respect to circulatory diseases, long-term contraceptive users and heavy smokers, included the vast majority of middle-aged women with impaired plasminogen activator activity.

*Key words:* Fibrinolysis, oral contraceptives, cigarette smoking

The plasminogen activators in the vessel wall are released by various stimuli to the blood stream, where they play a significant role in the defence system against spontaneous formation of thrombi in the vascular bed (15). Low fibrinolytic activity in the vessel wall or in plasma upon stasis has been demonstrated in patients with acute and recurrent thrombosis (5) as well as in patients with factors predisposing to thromboembolic disease (16).

In addition to vasoactive endogenous hormones such as adrenalin, bradykinin and vasopressin, several hormonal steroids influence the activity of plasminogen activators (16). Decreased activity has been reported during pregnancy (25), and after treatment with prednisolone (4) and ethinylestradiol (26). Increased activity has been demonstrated after treatment with the anabolic steroids stanozolol (24) and ethylestrenol (17), while treatment with progestogens such as chlormadinone and medroxyprogesterone acetate had no effects on the fibrinolytic activity (14, 27).

In women examined 6 months after a single thrombotic episode when using combined oral contraceptives (OC), low fibrinolytic activity in the vein wall (FAV) or in plasma upon stasis was demonstrated in about 30% of these patients without any demonstrable coagulation disorders (3). Furthermore, in a recent study on FAV in current OC users, impaired activity was found in the middle-aged pill users, and the smokers in this group frequently had low scores (8).

The purpose of the present study on middle-aged women was to investigate the possible influence on FAV of age, duration of OC usage and smoking habits. According to previous studies, the FAV reflects the activity of the tissue plasminogen activator in the endothelial cells lining the lumen and the *vasa vasorum* of the vessel wall (11).

## MATERIAL AND METHODS

The present series included 68 healthy, middle-aged women free from factors predisposing to thromboembolism (8), who were admitted to Huddinge Hospital for determination of FAV during 1983. Data on age, menstrual cycle, smoking habits and use of OC including duration and type, were registered prior to the sampling.

The 68 regularly menstruating women, aged 35 to 50 years, were divided into three groups:

*Group MP* consisted of 19 non-smoking contraceptive pill users (median age 40 years), all but 7 taking preparations containing 30 μg ethinylestradiol. Eleven women had taken OC continuously for more than 5 years, on average 10.5 years. The mean duration of OC treatment in the remaining 8 users was 2.1 years.

*Group MS* included 27 cigarette-smoking non-users (median age 40 years). Ten of these were light smokers with a daily consumption of less than 10 cigarettes, while the remaining 17 women smoked on average 20 cigarettes daily.

*Group MO* consisted of 22 healthy non-smokers (median age 39 years), who had never used OC, and served as control group.

In each patient a superficial vein on the dorsal foot was prepared by gentle dissection of the surrounding connective tissue after infiltration with 2 ml 0.5% prilocaine (Citanest$^{R}$, Astra). The vessel specimens, about 0.5 cm in length, were immediately frozen in liquid nitrogen and stored (−20°C) until analysis for fibrinolytic activity.

*Determination of the fibrinolytic activity in the vessel wall (FAV)*

FAV was assayed according to the semi-quantitative technique previously described by Pandolfi et al. (18). Briefly, five thin (8 μm) cross-sections of the vessel specimens were placed on a glass slide and covered with a thin, plasminogen-rich fibrin film, which was stabilized at room temperature (21°C) for half an hour. The slides were incubated in a moist chamber (37°C) for 0, 10, 20 and 30 min, after which they were fixed in formalin and stained. Fibrinolytic activity was reflected by clear lytic areas in the fibrin film and estimated blindly by the same laboratory assistant according to a four-graded scale. The FAV was expressed in arbitrary units as previously described (19).

*Statistics*

Non-parametric tests including the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U-test were used for the statistical evaluation of the results (23).

## RESULTS

The median values and distribution of the individual FAV scores within the three groups of healthy middle-aged women, i.e. non-smoking controls (MO), non-smoking pill users (MP) and smoking non-users (MS), are shown in Fig. 1. By analysis of variance a significant ($p<0.05$) difference between these three groups could be demonstrated. The median values of group MP and group MS were significantly lower than that of group MO. In the control group the scores were symmetrically distributed around the median value, and two-thirds of the scores were found within the normal range illustrated in Fig. 1.

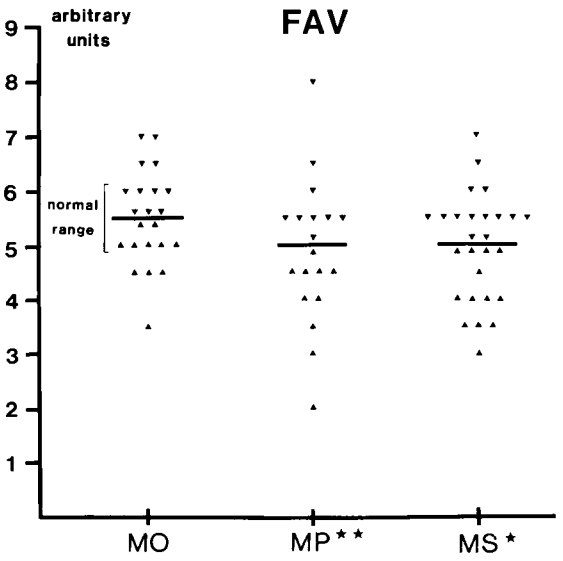


*Fig. 1.* Distribution of FAV scores in healthy middle-aged women with normal range and median values in non-smoking controls (MO), non-smoking pill users (MP) and cigarette-smoking non-users (MS). *$p<0.05$, **$p<0.01$.

The influence of the duration of OC treatment is evident from Table I, in which FAV has also been related to the daily cigarette consumption. The mean FAV in women using OC for more than 5 years was significantly decreased ($p<0.01$) when compared with users with shorter duration of OC treatment. Moreover, a significantly increased ($p<0.05$) frequency of low FAV values was found among the long-term users. However, the mean FAV in women using OC for less than 5 years was comparable to that in controls.

In the light smokers with a daily consumption of less than 10 cigarettes, the FAV was comparable to

Table I. *Fibrinolytic activity in vein walls of middle-aged pill users (MP) and cigarette smokers (MS) related to duration of OC use and smoking habits.*

| | Parameter | Number (n) | FAV | | Low FAV (≤4.5) |
|---|---|---|---|---|---|
| | | | Mean | (SD) | |
| *Group MP* | | | | | |
| Duration of OC use | ≤5 years | 8 | 5.7 | (1.1) | 13% |
| | >5 years | 11 | 4.2** | (1.0) | 73%* |
| *Group MS* | | | | | |
| Smoking habits | ≤10 cigs. | 10 | 5.3 | (1.0) | 10% |
| | >10 cigs. | 17 | 4.8 | (1.0) | 41% |

*$p<0.05$; **$p<0.01$

Table II. *Fibrinolytic activity in vein walls (FAV) in middle-aged women, related to age.*

| Group | Age 35–39 years FAV Mean±SD | Number (n) | Age 40–50 years FAV Mean±SD | Number (n) |
|---|---|---|---|---|
| MO[a] | 5.6±1.1 | (12) | 5.3±0.6 | (10) |
| MP[b] | 5.4±1.2 | (8) | 4.5±1.2 | (11) |
| MS[c] | 4.9±1.0 | (14) | 5.0±0.9 | (13) |

[a]Healthy controls; [b]pill users; [c]cigarette smokers

that of the control group. In heavy smokers, rather low mean FAV value and an increased frequency of low scores were recorded, although these differences were not statistically significant by comparison with the light smokers.

In the middle-aged women, no age-related variation in FAV was found, as seen in Table II, in which the mean FAV in the older group consisting of women aged 40 years or more has been compared with that of the younger group. The apparently lower FAV in the older pill users was due to an over-representation of women on long-term treatment.

## DISCUSSION

The close association between impaired plasminogen activator activity and increased risk of thromboembolic disease, initially reported in patients with recurrent idiopathic deep venous thrombosis, has been corroborated in several subsequent studies on fibrinolytic activity in plasma and vein walls (10, 17, 20). In recent studies on FAV, the activity of the arteries was closely correlated to that of the adjacent veins, and low activity was demonstrated in severely arteriosclerotic arteries (12, 13). Hence, low plasminogen activator activity may be associated with arterial disease, too, as proposed by Astrup (2). According to this theory, small deposits of fibrin lining tiny endothelial lesions may become incorporated in the arterial wall when there is insufficient release of plasminogen activators, thereby initiating the events leading to arteriosclerosis.

In the smoking non-users the majority of low FAV values were associated with heavy smoking, whereas light smoking had no effect on FAV. In a previous study on men aged 56 years, a slight decrease of the FAV was found in smokers (6). Immediately after the smoking of two cigarettes, elevated levels of tissue plasminogen activator have been demonstrated (1), and increased fibrinolytic activity in plasma following intravenous administration of nicotinic acid has been reported (21). In middle-aged heavy smokers, low resting levels and impaired release of tissue plasminogen activator have been demonstrated (1). Moreover, in rats exposed to cigarette smoke the decrease in FAV was associated with a corresponding increase in the plasma activity (9). Thus, the apparently dose-dependent reduction in FAV recorded in the middle-aged smoking non-users in this study agrees with the concept that cigarette smoking causes a gradual depletion of the plasminogen activator in the vessel wall, probably a result of the hazardous effect of carbon monoxide on the endothelial cell (1).

The very high frequency of low values observed in the long-term users of the present series is comparable to that recorded in women with factors predisposing to thromboembolism (8). Thus, the reduction in FAV in the middle-aged pill users appears to be exclusively an effect of long-term usage, since no difference in FAV was seen between the controls and women using OC for a few years. In OC users, increased plasma levels of tissue plasminogen activators have been reported recently (7). Hence, the present results accord with the concept that the FAV-reducing effect of long-term OC treatment may be caused by a combination of decreased synthesis and a depletion of the tissue plasminogen activators in the vessel wall (16).

In the middle-aged women the low FAV values were recorded mainly among long-term users and heavy smokers (Table I). Smoking and OC usage are also risk factors with respect to circulatory diseases. Thus, the reduced FAV and increased frequency of low values in these two well-known risk groups indicate that the impaired activity of the plasminogen activator may play an important etiological role in the pathogenesis of the vascular complications associated with OC usage and smoking (22).

We conclude that combined oral contraceptives are hardly suitable for women with low FAV with regard to the close association between this factor and vascular complications. However, in the present study on middle-aged women, the vast majority of low FAV values were recorded in women on long-term OC treatment, or in heavy smokers. Accordingly, screening of these two risk groups for FAV may be advisable prior to the prescription of combined oral contraceptives to the middle-aged woman.

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