Oral contraceptive use, thrombophilia and their interaction in young women with ischemic stroke

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To investigate the role of oral contraceptives and their interaction with thrombophilia in ischemic stroke, a case-control study on women with a first ischemic stroke when younger than 45 years was carried out. Oral contraceptives doubled the risk of ischemic stroke in the first 6-18 months of use and hyperhomocysteinemia increased the risk by 3.5-fold. Carriers of factor V Leiden or prothrombin G20210A were not found to have a statistically significant increased risk. The risk of ischemic stroke in oral contraceptive users was 13 times higher in women who were also carriers of factor V Leiden and 9 times higher in those who also had hyperhomocysteinemia.

Key words: thrombophilia, homocysteinemia, genetics, risk factors.

Haematologica 2006; 91:844-847

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Oral contraceptive (OC) use and inherited thrombophilia due to factor V Leiden or prothrombin G20210A and to deficiencies of antithrombin, protein C and protein S, are established risk factors for venous thromboembolism[1] including stroke due to cerebral vein thrombosis.[2,3] The use of OC also doubles the risk of ischemic stroke,[4-6] whereas the role of inherited thrombophilia is still controversial,[7-12] even though an association between ischemic stroke and prothrombin G20210A has been reported in the young.[10,11] In addition to conventional risk factors such as hypertension, hypercholesterolemia and diabetes, the metabolic abnormality hyperhomocysteinemia is a recognized risk factor for ischemic stroke,[13] also in the young.[14] This risk varies depending on the coexistence of multiple risk factors; for instance, hypertension and smoking enhance the risk associated with OC use.[5,15,16] Few data are available on the interaction between thrombophilia and OC in patients with ischemic stroke.[12] Considering that factor V Leiden, prothrombin G20210A and hyperhomocysteinemia are common in Caucasian populations,[1,13] and that OC are used by approximately 30% of women in Italy, we focused on the interaction of these risk factors on the risk of ischemic stroke in young women.

Design and methods

Patients

One hundred and nineteen unrelated Caucasian women of fertile age who were referred to the Thrombosis Center between January 1994 and June 2005 for thrombophilia screening after a first ischemic stroke were enrolled in this study. The median time elapsed between the stroke and blood sampling was 6 months (range 1 month-10 years); 75% of patients had blood sampled within 2 years and 64% within 1 year after the event. Clinical records were reviewed and when the type of stroke was not specified, neurologists who took care of the patients during the acute phase were contacted. Eleven patients were excluded because of incomplete medical documentation and three because of previous venous thrombosis. Therefore, 105 patients with a first, objectively confirmed ischemic stroke were included in the study. The clinical diagnosis was objectively confirmed by computed tomography scans in 41 patients, magnetic resonance or magnetic resonance angiography in 61, and intra-arterial angiography in three. All patients underwent a cardiological assessment and transthoracic echocardiography (in 76% of patients completed by transesophageal examination) and Doppler examination of the neck vessels.

Two hundred and ninety-three healthy, unrelated, Caucasian women of fertile age were chosen from the whole population of controls made of partners and friends who accompanied patients to the Center in the same period as patients and agreed to be investigated. Previous thrombosis in the controls was excluded using a validated structured questionnaire.[17]

The presence, at the time of stroke for patients and at the time of blood sampling for controls, of hypertension, hypercholesterolemia, diabetes mellitus, smoking (at least five cigarettes daily) and obesity was recorded. Likewise the type and duration of

OC intake was recorded. Women were considered to be on OC if they had taken them within the 2 weeks before referral. No women had abnormal liver or renal function, overt autoimmune or neoplastic diseases, and none was pregnant. The study was approved by the Institutional Review Board of the University of Milan and all women gave their informed consent to the study. Thrombophilia screening included DNA analysis for factor V Leiden and prothrombin G20210A, functional and/or antigenic assays for antithrombin, protein C and protein S, and assays of plasma total homocysteine after overnight fasting and 4 hours after an oral methionine load (3.8 g/m^2 body surface area).[2] Hyperhomocysteinemia was diagnosed as previously described.[18]

The Student's t-test was performed to compare continuous variables, after testing for normality the Kolmogorov-Smirnov test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the association between stroke, thrombophilia and OC intake. Using a logistic regression model, OR were adjusted for the stratification variable (age) and for possible confounders (educational level, hypertension, hypercholesterolemia, obesity, smoking), and the interaction between thrombophilia and OC use was evaluated. All statistical tests were two-sided with an α level of 0.05. The analyses were performed using the S-PLUS package, version 6.03 (1988-2001 Insightful Corp, Lucent Technologies, Inc.).

Results and Discussion

Table 1 shows the general characteristics of the study population and the subtypes of stroke according to the TOAST classification.[19] As shown in Table 2, OC were more often used by patients than by controls with an OR of 2.3 (95% CI 1.4-3.8). OC were of third generation (desogestrel or gestogene as the progestin) in 58% of patients and in 76% of controls. All stroke victims on OC had their stroke within the first 18 months of OC use, 86% within the first 12 months and 75% within the first 6 months. Deficiencies of antithrombin, protein C or protein S, as well as prothrombin G20210A were not associated with an increased risk of stroke, whereas factor V Leiden gave a non-statistically significant OR of 2.6. Hyperhomocysteinemia was significantly associated with a 3.5-fold increased risk of ischemic stroke; this association remained when only patients referred within one year after the stroke and those who were not taking barbiturates (which may affect homocysteine levels) at the time of blood sampling were considered (*data not shown*).

Table 3 shows the interactions between OC use and factor V Leiden, prothrombin G20210A or hyperhomocysteinemia. After stratification of the study population according to the use or not of OC and the presence or absence of thrombophilia, factor V Leiden increased the risk associated with the use of OC from 2- to 13-fold and that of hyperhomocysteinemia from 2- to 6-fold; both these increases were statistically significant. No increased risk was observed as a consequence of combined presence of OC use and prothrombin G20210A. When patients were divided into those with stroke of undetermined etiology (n=55) and stroke of known cause (n=50), a slightly more pronounced association with OC use (OR 2.8, 95%CI 1.5-5.5) and hyperhomocysteinemia (OR 3.8, 95%CI 1.8-8.0) was observed in the former group than in the latter (OR 1.9, 95% CI 0.9-3.9 and 3.0, 95%CI 1.1-6.7, respectively). Interaction analyses could be done in the two groups only for OC use and hyperhomocysteinemia which, in combination, gave similar OR of 7.8 (95%CI 1.7-36.5) for stroke of undetermined etiology and 8.0 (95%CI 2.3-27.2) for stroke of known etiology. In two previous studies with

Table 1. Characteristics of the study population and presence of conventional risk factors for ischemic stroke.

	Patients	*Controls*	*p*
Number	105	293	
Stroke etiology, n. (%):			
large-artery atherosclerosis*	13 (12)		
cardioembolism†	24 (23)		
small-vessel occlusion	8 (8)		
vasculitis	5 (5)		
undetermined	55 (52)		
Age (SD), y‡	34.7 (9.1)	34.9 (8.6)	>0.05
Body mass index (SD), Kg/m^2‡	23.1 (3.8)	22.5 (4.0)	>0.05
Educational level, n. (%):			
grade school	8 (8)	6 (2)	<0.05
high school	27 (26)	64 (22)	
college	54 (51)	129 (44)	
university	16 (15)	94 (32)	
Conventional risk factors, n. (%):			
hypertension	13 (12)	9 (3)	< 0.001
hypercholesterolemia	11 (10)	1 (0.3)	< 0.001
obesity	5 (5)	13 (4)	>0.05
smoking	28 (27)	61 (21)	>0.05

**carotid or anterior system (n=10) vertebrobasilar system (n=3); †patent foramen ovale (n=15), atrial fibrillation (n=5), atrial septal or left ventricular aneurysm (n=4); ‡age and body mass index are expressed as means (SD).*

Table 2. Association between oral contraceptive use and thrombophilia markers in ischemic stroke.

Risk factor, n. (%)	*Patients*	*Controls*	*Odds ratio (95%CI)*	*Odds ratio (95%CI)**
Oral contraceptive use	43 (41)	67 (22)	2.3 (1.5-3.8)	2.3 (1.4-3.8)†
Thrombophilia marker				
factor V Leiden‡	6 (6)	7 (2)	2.5 (0.8-7.5)	2.6 (0.8-8.0)
prothrombin G20210A‡§	5 (5)	15 (5)	0.9 (0.3-2.6)	0.9 (0.1-11.2)
AT, PC or PS deficiency¶	1 (1)	5 (2)	0.6 (0.06-4.7)	0.5 (0.06-4.6)
hyperhomocysteinemia§¶	25 (24)	26 (8)	3.2 (1.8-5.9)	3.5 (1.9-6.4)

**adjusted for age; †also adjusted for educational level, hypertension, hypercholesterolemia, obesity and smoking; ‡all individuals with mutations were heterozygous for the mutation; §one patient had prothrombin G20210A and hyperhomocysteinemia; ¶one control had antithrombin deficiency and hyperhomocysteinemia and another had protein S deficiency and hyperhomocysteinemia.*

Table 3. Interaction between oral contraceptive use and thrombophilia due to factor V Leiden, prothrombin G20210A and hyperhomocysteinemia.

Factor V Leiden	*Oral contraceptive use*	*Odds Ratio (95%CI)**
No	No	1(Ref.)
No	Yes	2.1 (1.3-3.6)
Yes	No	1.4 (0.3-7.3)
Yes	Yes	12.9 (1.3-133.7)
Prothrombin G20210A		
No	No	1(Ref.)
No	Yes	2.2 (1.3-3.7)
Yes	No	0.5 (0.1-2.5)
Yes	Yes	3.1 (0.4-23.3)
Hyperhomocysteinemia		
No	No	1(Ref.)
No	Yes	2.4 (1.4-4.2)
Yes	No	3.4 (1.5-7.6)
Yes	Yes	6.2 (1.7-22.0)

**adjusted for age, educational level, hypertension, hypercholesterolemia, obesity and smoking.*

a similar design but different criteria for selecting patients factor V Leiden was not associated with an increased risk of ischemic stroke in the young, with the possible exception of stroke of undetermined etiology,[8] and prothrombin G20210A was not a risk factor for ischemic stroke or transient ischemic attack.[9] In this study carried out in a new cohort of young women, OC use was associated with a 2-fold increased risk of ischemic stroke. The risk was increased 3.5-fold in the presence of hyperhomocysteinemia and 2.6-fold (which was not statistically significant) in the presence of factor V Leiden, whereas the prothrombin G20210A mutation and deficiencies of antithrombin, protein C or protein S did not increase the risk. OC use and hyperhomocysteinemia when present together conferred a 6-fold increased risk of ischemic stroke, whereas OC use and factor V Leiden increased the risk from approximately 2-fold for each factor alone to 13-fold. The risk associated with OC use was slightly higher for stroke of undetermined etiology. These findings are in agreement with a recent observation of an increased risk of ischemic stroke in women who use OC and who are also carriers of factor V Leiden or the 677TT polymorphism in the gene coding for methylentetrahydrofolate reductase (MTHFR).[12] The *MTHFR* 677TT polymorphism, together with low levels of serum folate, is a determinant of hyperhomocysteinemia.[13] The main differences between this and that study are that we measured the most appropriate risk factor for thrombosis and atherosclerosis, i.e., total plasma homocysteine, and that we were able to evaluate two groups of ischemic strokes, those of undetermined etiology and those of known etiology. In accordance with a couple of studies[9,12] but at variance with others,[10,11] we observed no increased risk of ischemic stroke in women with prothrombin G20210A. This difference is likely to be due to different sample size or selection criteria of the study population.

Some limitations of this study need to be addressed. We investigated a highly selected population of survivors of ischemic stroke referred to our Center for thrombophilia screening. As expected, women with the rare subtype of unexplained ischemic stroke or those with a positive family history of thrombosis (60% of our series) were preferentially referred. Patients who died from the stroke were lost, but due to the low mortality rate in the young[20] this is unlikely to have to an underestimation of the risk. Finally, some interaction analyses on OC and thrombophilia should be interpreted with caution because of the relatively large confidence intervals after stratification of the study population.

This study may have clinical implications for the appropriateness of thrombophilia screening in young women with ischemic stroke, but whether or not hyperhomocysteinemia should be searched for in young women on OC in order to prevent ischemic stroke remains to be established by prospective investigations. Further studies of more women are needed to elucidate the role of thrombophilia and to assess whether or not costs of DNA analysis for factor V Leiden will balance benefits of large-scale screening.

IM: conception and design of the study; drafting article; final approval; TB: analysis and interpretation; revising article; final approval; IB: acquisition of data; revising article; final approval; SDD: acquisition of data; revising article; final approval; PMM: critically reviewed article; final approval. The authors declare that they have no potential conflicts of interest.

Manuscript received February 20, 2006. Accepted April 11, 2006.

References

1. Martinelli I. Risk factors in venous thromboembolism. Thromb Haemost 2001;86:395-403.
2. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. N Engl J Med 1998;338:1793-7.
3. de Bruijn SFTM, Stam J, Koopman MMW, Vandenbroucke JP. Case-control study of risk of cerebral sinous thrombosis in oral contraceptive users who are carriers of hereditary prothrombotic conditions. The Cerebral Venous Sinous Thrombosis Study Group. Br Med J 1998;316:589-92.
4. Gillum LA, Mamidipudi SK, Johnston SC. Ischemic stroke risk with oral contraceptives. A meta-analysis. JAMA 2000;284:72-8.
5. Kemmeren JM, Tanis BC, van den Bosch MA, Bollen EL, Helmerhorst FM, van der Graaf Y, et al. Risk of arterial thrombosis in relation to oral contraceptives (RATIO) study: oral contraceptives and the risk of ischemic stroke. Stroke 2002;33:1202-8.
6. Lidegaard O, Kreiner S. Contraceptives and cerebral thrombosis: a five-year national case-control study. Contraception 2002;65:197-205.
7. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutations in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 1995;332:912-7.
8. Landi G, Cella E, Martinelli I, Tagliabue L, Mannucci PM, Zerbi D. Arg506Gln factor V mutation and cerebral ischemia in the young. Stroke 1996;27:1697-8.
9. Martinelli I, Franchi F, Akwan S, Bettini P, Merati G, Mannucci PM. The transition G to A in the 3'-untranslated

region of the prothrombin gene is not associated with cerebral ischemia. Blood 1997;90:3806.
10. De Stefano V, Chiusolo P, Paciaroni K, Casorelli I, Rossi E, Molinari M, et al. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. Blood 1998;91:3562-5.
11. Aznar J, Mira Y, Vaya A, Corella D, Ferrando F, Villa P, et al. Factor V Leiden and prothrombin G20210A mutations in young adults with cryptogenic ischemic stroke. Thromb Haemost 2004;91:1013-4.
12. Slooter AJC, Rosendaal FR, Tanis BC, Kemmeren JM, Van der Graaf Y, Algra A. Prothrombotic conditions, oral contraceptives, and the risk of ischemic stroke. J Thromb Haemost 2005; 3: 1213-7.
13. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. Thromb Haemost 1999;81:165-76.
14. Madonna P, De Stafano V, Coppola A, Cirillo F, Cerbone AM, Orefice G, et al. Hyperhomocysteinemia and other inherited prothrombotic conditions in young adults with a history of ischemic stroke. Stroke 2002;3: 51-6.
15. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Ischaemic stroke and combined oral contraceptives: results of an international, multicentre, case-control study. Lancet 1996;348:498-505.
16. Lothar AJ, Heinemann MAL, Spitzer WO, Thorogood M, Guggenmood-Holzmann I, Bruppacher T. Thromboembolic stroke in young women. A European case-control study on oral contraceptives. The Transnational Research Group on Oral Contraceptives and the Health of Young Women. Contraception 1998; 57:29-37.
17. Frezzato M, Tosetto A, Rodeghiero F. Validated questionnaire for the identification of previous personal or familial venous thromboembolism. Am J Epidemiol 1996;143:1257-65.
18. Zighetti ML, Cattaneo M, Falcon CR, Lobardi R, Harari S, Savoritto S, et al. Absence of hyperhomocysteinemia in ten patients with primary pulmonary hypertension. Thromb Res 1997;85: 279-82 .
19. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtypes of acute ischemic stroke. Definition for use in a multicenter clinical trial. The TOAST Investigators. Stroke 1993;24:35-41.
20. Sarti C, Stegmayr B, Tolonen H, Mahonen M, Tuomilehto J, Asplund K. Are changes in mortality from stroke caused by changes in stroke event rates or case fatality? Results from the WHO MONICA Project. Stroke 2003;34:1833-40.