Activated protein C resistance in young adults with central retinal vein occlusion

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Abstract

Background—Central retinal vein occlusion is a disease that is most common in old people. While the disease in old people often is associated with atherosclerosis, hypertension, diabetes, or glaucoma, this is much less evident in young people. However, a new defect in the anticoagulant system has recently been discovered, activated protein C resistance. This hereditary defect may well be associated with central retinal vein occlusion, and so this factor was analysed in patients younger than 50 years with a history of central retinal vein occlusion.

Methods—Blood samples were obtained from 31 patients younger than 50 years with a history of central retinal vein occlusion, and analysed for activated protein C resistance with standard clinical laboratory methods.

Results—In this material 26% of all the patients and 36% of the patients younger than 45 years were resistant to activated protein C. The normal incidence of activated protein C resistance is 2–7%.

Conclusion—Activated protein C resistance seems to be the most common known cause of central retinal vein occlusion in young people.

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Central retinal vein occlusion (CRVO) is a disease that is fairly common in elderly people. Glaucoma, hypertension, atherosclerosis, and diabetes are factors that are well known to be associated with the disease.¹ However, in young people it has been impossible to find a triggering factor. Retrospective studies have been done in young patients with a history of CRVO with the intention of trying to find possible predisposing factors for the thrombosis but nothing conclusive has emerged.^{2–5} In the majority of cases, a reason for the CRVO was not found. In large amounts of materials from patients with a diagnosis of thrombophilia, hereditary deficiencies in the coagulation system have been found in only 4–6%.^{6,7}

The coagulation system is a complex process involving a cascade of reactions resulting in the conversion of prothrombin to thrombin, which finally converts fibrinogen into fibrin. However, there is also the anticoagulant system with its most important factors – protein C, protein S, and antithrombin III. Normally there is a balance between these two systems, which inhibits the formation of thrombosis.⁸ If there is an injury on the endothelium, the balance changes in favour of coagulation, and a

thrombus is formed as a start of the healing process. In order to avoid excessive clot formation, activation of the coagulation also activates the anticoagulation system. When the clot has sealed off the endothelial injury, the coagulation reaches intact endothelium on which there is a protein, thrombomodulin, which binds thrombin and inactivates it. This complex of thrombin with thrombomodulin activates protein C, which inhibits coagulation by degrading coagulation factors Va and VIIIa.⁹

In 1992 Dahlbäck *et al* discovered that some young patients with thrombosis had a resistance to activated protein C (APC).^{10,11} Although APC was added to the patient's plasma it did not result in the normal inhibition of the coagulation. Thus, there seemed to be a resistance in the patient's plasma to APC. Later they were able to show that APC resistance was due to a selective defect in the anticoagulant function of factor Va7. The defect is a point mutation that has been localised to the locus for the factor V gene on chromosome 1,^{12–14} and it is dominantly inherited.⁹

The prevalence of this mutation in a normal population has, in different investigations, been found to be between 2 and 7%.^{9,13,15,16} Several materials from young patients with a history of thrombosis, mainly in the leg, have been examined for APC resistance and the prevalence has been found to vary between 21% and 52%, with the higher values if there is a history of thrombophilia in the family.^{9,13,16,17}

In order to examine the prevalence of resistance to APC in patients with unexplained CRVO, we have examined material from 31 such patients aged below 50 years.

Patients and methods

PATIENTS

The population sample was recruited from the patient records of the eye clinics of the Lund University Hospital and the Örebro Medical Center Hospital in Örebro, Sweden. Of the 34 patients found, blood samples were obtainable from 31, together with their history of smoking, oral contraceptives, and other factors predisposing to thrombosis as well as their family history of thrombosis. The follow up time was between 1 and 6 years (median 2 years).

The material comprised 16 (52%) males and 15 (48%) females. At the time they had their CRVO their median age was 40 years (average 39.0; range 19–48). Five patients had a family history of thrombosis. One patient had antibodies against the lupus anticoagulants antigen and one patient had a positive rheumatoid

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factor. In the rest of the patients, all laboratory tests including haemoglobin, erythrocyte sedimentation rate, erythrocyte volume fraction, activated partial thromboplastin time (APTT), and prothrombin time were normal.

METHODS

The analysis of resistance to APC was carried out at the department of clinical chemistry, Malmö, and at the department of clinical chemistry, Örebro, Sweden. The method is the same as that described by Dahlbäck *et al.*¹⁰ Blood was collected in vacutainer tubes containing 0.5 ml of 0.12 M sodium citrate and centrifuged at 2000 *g* for 20 minutes. The plasma was frozen and stored at -70°C until it was analysed. In the APC resistance test, 0.1 ml of plasma was incubated with 0.1 ml of APTT reagent (APTT automated or Platelin (LS, Organon Teknika)) for 5 minutes at 37°C before coagulation was initiated by the addition of 0.1 ml of an APC/ CaCl_2 mixture. The coagulation time was recorded with an MLA 900C apparatus. Each plasma sample was also analysed in parallel without the addition of APC to the CaCl_2 solution, and the APC dependent prolongation of the clotting time was calculated. The APC ratio was estimated by dividing the clotting time obtained with the APC/ CaCl_2 solution by the clotting time obtained with CaCl_2 alone. When a ratio below 2 SD (2.8 in this study) of a set of reference analyses was obtained, the patient was considered to be resistant to APC.

Results

Resistance to activated protein C was seen in eight of the 31 patients (26%; 95% confidence limits 12–45%). The average APC ratio in these eight patients was 1.95 (range 1.5–2.4) and 3.71 (range 3.1–4.4) among the normal subjects. The protein C induced prolongation of their coagulation time was always either normal or clearly pathological, and no borderline cases were encountered. In patients younger than 45 years, the same eight (36%; 95% confidence limits 17–59%) were resistant to APC. In the normal population, 2–7% show such resistance,^{9 13 15 16} which means that in the present cohort of young patients with a CRVO, there is an overrepresentation of more than four times of APC resistance in the total population sample, and perhaps as much as 18 times in patients younger than 45 years.

Among the eight APC resistant patients, 50% had a visual acuity that was 0.1 (=20/200) or worse at the end of the follow up time. On the other hand, only five patients (22%) among the 23 who did not show APC resistance had a visual acuity of 0.1 (20/200) or worse. However, the difference is not statistically significant.

Discussion

This study of 31 patients shows that activated protein C resistance is much more common in patients with central retinal vein occlusion than

in the normal population. The increase is at least fourfold with the lower 95% confidence limit being about 12%, which is well above the range found in other published population studies.^{9 13 15 16} APC resistance is a very recently discovered aberration^{9 10} and no studies have previously been published that show its correlation with CRVO. However, Ie *et al.*¹⁸ recently presented three patients with bilateral occlusions who also showed APC resistance.

The effect of APC is measured by noting the increase in coagulation time caused by the addition of a known amount of APC. The analysis discriminates readily between normal and affected patients.⁹ Similarly, we did not see any borderline cases in the present study, making it easy to classify the patients as having or not having resistance to APC.

In previous laboratory investigations, only 4–6% of all thrombosis patients have been found to have some hereditary deficiency in their coagulation system, irrespective of the site of the lesion.^{6 7} However, APC resistance was not looked for in these studies. As seen in the present work, APC resistance appears to be at least four to five times more common in young patients with CRVO than any other known inherited deficiency of anticoagulant proteins. Thus, APC resistance is the most common known cause of CRVO in young patients. We therefore suggest that an analysis of APC resistance should be included in the examination of every young patient with a thrombosis.

Since 2–7% of the population are resistant to APC, but the yearly incidence of thrombosis in patients is much less in patients up to 50 years of age, it seems that APC resistance alone is not enough to cause a thrombosis. There have to be other factors that trigger it. Examples of such factors are smoking, immobilisation, oral contraceptives, and operations.¹¹ Such factors were looked for in this study, but no significant results were found, probably because of the small number of patients.

APC resistance is dominantly inherited and the question arises whether relatives of the patients should be examined in order to find those who are resistant to APC. The benefit of this is that they can be told they are more susceptible than others to thrombosis and that it is important they receive some kind of thromboprophylaxis when they are in a situation that predisposes to thrombosis, such as an operation. They can also be told that it is even more dangerous for them to smoke than for other people, and that women should discuss their use of oral contraceptives with their gynaecologist. Svensson *et al.*⁹ made a family study of 177 people in 34 families in which one family member was resistant to APC. He found that 45% were resistant to protein C and that 49 people (27%) had a history of thrombosis. In these families the probability that an APC resistant person would be free of thrombosis at the age of 45 was only 59% compared with 97% for a relative without APC resistance.⁹ We feel it is important to inform the patient that APC resistance is a dominantly inherited disease and that

there is a significant risk that other members of the family have the same gene error. It is then up to the individual patient together with his or her physician to decide whether it is appropriate to examine other family members.

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