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Effect of danazol on the biochemical abnormality of inherited antithrombin III deficiency

AJ FAIRFAX, RM IBBOTSON

From the Department of Thoracic Medicine, Staffordshire General Infirmary, Stafford, and the Department of Haematology, Central Pathology Laboratory, Hartshill, Stoke on Trent

ABSTRACT Seven members of a family affected by hereditary antithrombin III deficiency were identified. The disorder was associated with recurrent spontaneous episodes of phlebitis, deep venous thrombosis, and pulmonary embolism in middle age. Danazol, a 17-alkyl derivative of ethinyl testosterone, which has been used to treat other antiprotease deficiency states, was assessed in the management of two men with antithrombin deficiency. In a dose of 600 mg a day danazol appeared to correct the antithrombin deficiency. This drug may provide a useful adjunct to anticoagulant treatment, particularly before surgery.

Inherited antithrombin III deficiency (hereditary thrombophilia) is a rare disorder, first described by Egeberg in 1965,[1] which is characterised by recurrent, sometimes fatal, deep vein thrombosis and pulmonary embolism. When recognised, the condition is usually treated by long term anticoagulation. The control mechanisms that inhibit blood coagulation are vital for homeostasis. The activation sequence in the coagulation cascade contains negative feedback effects that tend to limit thrombin formation.

Antithrombin is one of the protease inhibitors present in blood and its main role is to inactivate thrombin. Antithrombin also inactivates all the clotting enzymes except factor VIIa. Heparin accelerates the reaction between antithrombin and the clotting enzymes.

The structure of antithrombin has recently been determined and is very similar to that of α_1 antitrypsin.[2] They are believed to have evolved from a common ancestral protein—a mutation resulting in the substitution of a single amino acid (arginine for methionine) in the α_1 antitrypsin molecule converts this from an inhibitor of elastase to an inhibitor of thrombin.[3] Molecular heterogeneity occurs in inherited antithrombin III deficiency as in α_1 antitrypsin deficiency.[4]

Address for reprint requests: Dr AJ Fairfax, Department of Thoracic Medicine, Staffordshire General Infirmary, Stafford ST16 2PA.

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Synthetic androgens such as danazol are known to induce the formation and release of various protease inhibitors, including Cl-esterase inhibitor[5] and α_1 antitrypsin.[6] The mechanism underlying this effect is unknown. The aim of this study was to determine whether danazol would correct the biochemical abnormality in inherited antithrombin III deficiency.

Methods

ANTITHROMBIN III ASSAY

Blood was mixed with citrate anticoagulant (3.8% trisodium citrate 1 vol: 9 vol blood). After centrifugation the separated plasma was stored at −70°C. Antithrombin III antigen was measured by radial immunodiffusion,[7] Behringwerke Norpartigen plates being used (Behringwerke AG, Marburg, West Germany). Biological antithrombin III activity was assessed as heparin cofactor with chromogenic substrate S 2238 (Coa test, Kabi AB, Stockholm). This functional assay using a synthetic chromogenic substrate has been shown to give a sensitive and precise measure of antithrombin activity in plasma.[8] The laboratory normal ranges for functional antithrombin III and antithrombin III antigen are 84–116% and 72–128%. The results are conventionally expressed as percentages of the activity present in reference plasma. The Coa test for functional antithrombin III activity has a coefficient of variation of 2.1 (n = 35) and the radial immunodiffusion method a coefficient of variation of 2.3 (n = 14). Warfarin anticoagulant control was monitored with the Manchester comparative

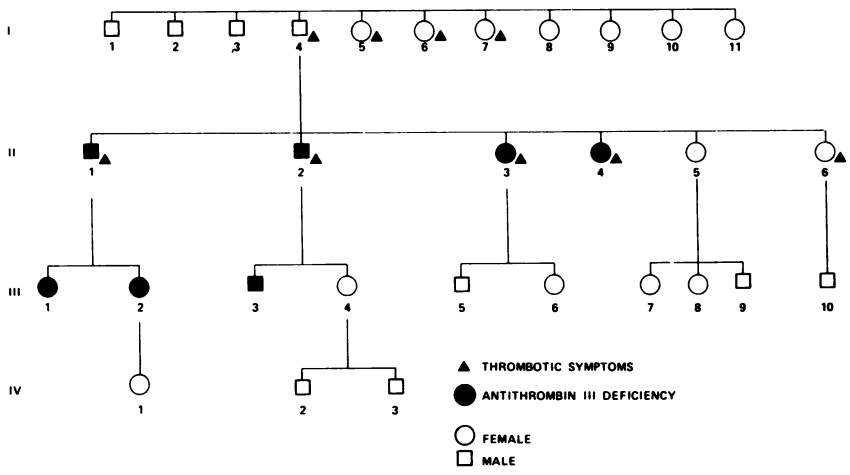

Fig 1 *Pedigree of the family showing an autosomal dominant mode of inheritance, with antithrombin III deficiency found in seven cases. In addition, four deceased members of generation I had probably been affected.*

reagent and adjusted to the therapeutic range 1.8–3.0.

Case reports and results

The generations of the affected family are shown in figure 1, designated by Roman numerals.

THE CASES

Case II 1 The index patient, a 56 year old farmer, was well until the age of 41, when he developed pain and stiffness in the left leg. One year later he became breathless and experienced bilateral pleuritic pain. Investigations showed multiple pulmonary emboli associated with small pleural effusions. He was treated with warfarin for seven months. He remained well for 10 years and then had a spontaneous right deep venous thrombosis, confirmed by venography. Warfarin was given for eight months and he was then prescribed long term aspirin treatment. Two years later, when he was 54, his left leg swelled intermittently and he experienced further pleuritic pain. Multiple pulmonary emboli were demonstrated on a perfusion lung scan. These subsequently resolved. Antithrombin III deficiency was diagnosed and he was given long term anticoagulation treatment with warfarin. Currently he complains of intermittent aching in the left calf, varicose eczema, and varicose veins. His father had symptoms from leg veins and a familial tendency to phlebitis had been recognised by the family. Other members of the preceding generation (I) had had symptoms suggesting antithrombin deficiency but were now dead (fig 1).

Case II 2 A 59 year old farmer, brother of the propositus, presented at the age of 48 with pleuritic pain and a swollen right calf. One month later the left calf also became swollen and venography showed a deep venous thrombosis. He was treated for seven months with warfarin, but when this was discontinued the leg symptoms recurred. He was subsequently treated with warfarin indefinitely. He was found to have hyperlipidaemia at the age of 51 and was started on a low cholesterol diet and clofibrate. A further episode of suspected pulmonary embolism occurred when he was 55. He suffered two myocardial infarctions at the age of 57 and again two years later. The right leg swells intermittently and there are visible varicose veins and varicose eczema.

Other family members The siblings in generation II were all traced and three had varicose veins or thrombotic symptoms or both. Two of these (II 3 and II 4) were found to have antithrombin III deficiency. All those with either deep venous thrombosis or pulmonary embolism had developed their first symptoms after the age of 40 years. All the offspring from the affected individuals in generation II were examined except for one who had died in an accident. Three of the 10 who were seen in this generation (III) also had antithrombin III deficiency; two of them (aged 23 and 25) were symptomless and one had varicose veins (fig 1).

RESULTS OF INVESTIGATIONS

In the seven affected family members the functional assay gave values of 33–69% for antithrombin III activity (normal range 84–116%) with a mean (SD) value of 56% (11%). The immunoassay results were usually slightly higher, with a mean (SD) of 66%

Family members with antithrombin III deficiency

No	*Sex*	*Age (y)*	*Clinical features*	*Antithrombin activity (%)*		*Treatment*
				Chromogenic	*Antigenic*	
II 1	M	56	Recurrent DVT, pulmonary embolism, varicose veins	33–75	56–68	Warfarin
II 2	M	59	Recurrent DVT, pulmonary embolism, varicose veins, coronary thrombosis	36–57	54–62	Warfarin
II 3	F	63	Recurrent DVT	53–69	68	Warfarin
II 4	F	48	Recurrent phlebitis, varicose veins	54–68	65–66	
III 1	F	27	Varicose veins	66–76	78–102	
III 2	F	25	None	37–40	43–65	
III 3	M	23	None	58	60	

DVT—deep vein thrombosis.

(11%), the normal range being 72–128% (table). The levels of functional antithrombin III activity showed a clear separation between affected and normal family members, the latter having values of 96–147% (mean 122%), with similar values of 90–110% (mean 101%) estimated by immunoassay. A raised serum cholesterol concentration of 7.4–9.2 mmol/l (285.7–355.2 mg/100 ml) (normal range 4.0–6.5 mmol/l (154.4–251.0)) was found in four subjects with antithrombin III deficiency; two of these also had fasting hypertriglyceridaemia, with triglyceride concentrations of 3.5–5.3 mmol/l (309.7–469.0 mg/100 ml) (normal range 0.5–2.0 mmol/l (44.2–177.0 mg/100 ml)).

TREATMENT

With informed consent, patients II 1 and II 2 were treated with danazol in divided doses by mouth of up to 600 mg/day. This resulted in a rise in plasma antithrombin III activity within seven days of starting treatment as assessed by both assays (figs 2 and 3). No changes occurred in blood urea or electrolyte concentrations or in the results of liver function tests (including total protein and albumin), but the α_1 antitrypsin activity increased during the periods of danazol treatment. Patient II 1 received two courses of danazol. The daily dose was progressively increased: 400 mg/day was sufficient to bring the chromogenically assayed antithrombin III within the normal range (fig 3) and 600 mg/day resulted in a further increase. The only side effects reported were mild headaches in one man and a sene of wellbeing in the other. Warfarin, which is known not to affect antithrombin III levels,[9] was continued during the danazol treatment to keep the prothrombin ratio within the therapeutic range. The dose of warfarin had to be reduced in both cases to prevent excessive anticoagulation.

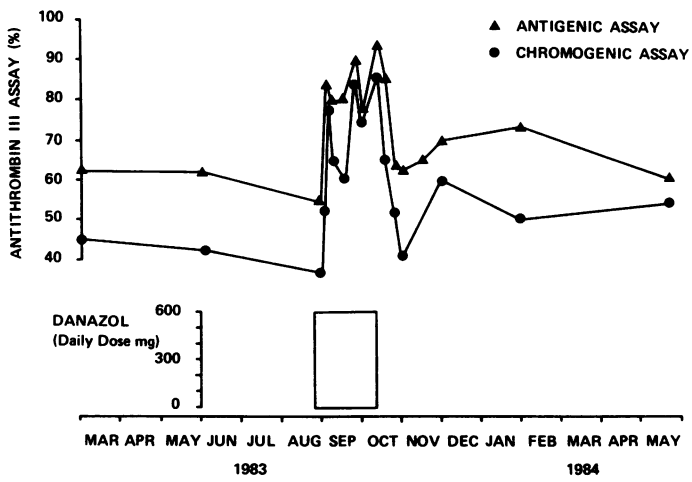

Fig 2 *Response of antithrombin III level to danazol treatment (patient II 2).*

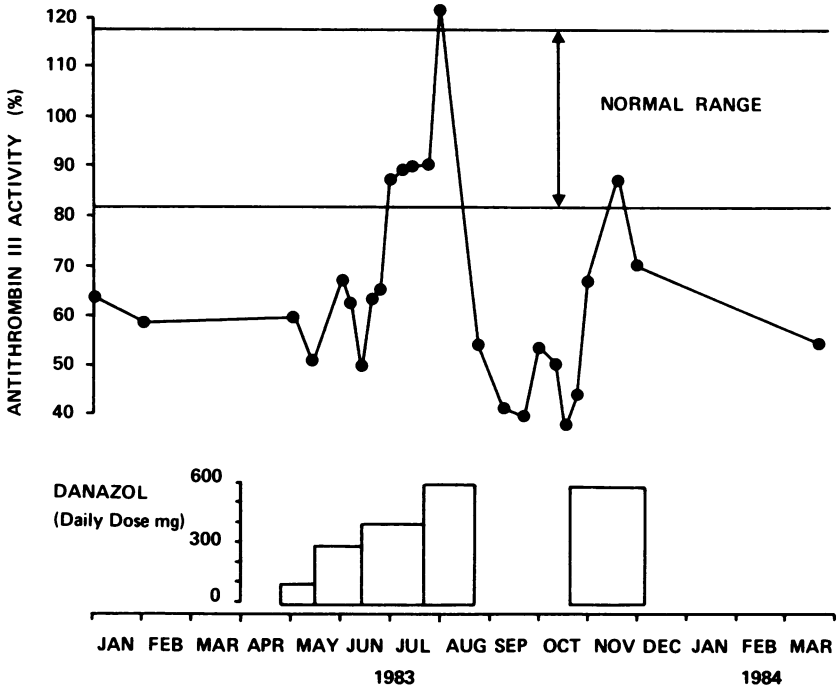

Fig 3 *Response of antithrombin III level to increasing doses of danazol and to a second period of treatment (patient II 1).*

Discussion

The cases in this family are similar to those in other families with hereditary antithrombin III deficiency.[1 9] The mode of inheritance is typically autosomal dominant as in this family. Recurrent deep venous thrombosis and pulmonary embolism later in life are characteristic of the condition. Varicose veins, probably secondary to previous deep venous thrombosis, were found in most of the affected members of this family, who had themselves recognised this to be a familial characteristic. Hyperlipidaemia may occur with antithrombin III deficiency but recent evidence suggests that this is a chance association.[9 10]

Anabolic steroids have previously been observed to produce a modest augmentation of plasma antithrombin III activity,[11-13] in contrast to oestrogen-progesterone contraceptive preparations, which slightly depress antithrombin III activity.[14] We are not aware of any previous reports of danazol in the treatment of antithrombin III deficiency, despite the drug's usefulness in other protease deficiency states and the recent interest in the possible use of danazol to augment factor VIII and factor IX activity in haemophilia and Christmas disease.[15] In the two patients described a therapeutic dose of danazol reversibly corrected the antithrombin III deficiency without appreciable side effects. Alpha$_1$ antitrypsin activity also increased, which is of interest in view of its very close molecular similarity to antithrombin III.[2] In the present study danazol produced normalisation of antithrombin levels, in contrast with the modest changes reported with other drugs.[11-13]

For long term treatment warfarin is probably the treatment of choice. Danazol offers a possible alternative where warfarin is sometimes contraindicated—for example, before major surgery. The drug may also prove a useful adjuvant to anticoagulation where venous thrombosis occurs despite warfarin treatment.

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