

Long-term treatment of hereditary angioedema with attenuated androgens: A survey of a 13-year experience

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Fifty-six patients affected with hereditary angioedema have been followed during long-term prophylaxis with attenuated androgens. The treatment was started in patients who had one or more severe attacks per month. In 24 patients, the therapy lasted for more than 5 years. The minimal effective dose usually did not exceed 2 mg/day of stanozolol or 200 mg/day of danazol. Only in two patients were these doses not sufficient to achieve the complete disappearance of symptoms. Irregular menstruation, but rarely amenorrhea, was the only significant side effect. One patient had to stop the therapy because of laboratory signs of hepatic cell necrosis. In one patient, danazol was administered during the last 8 weeks of pregnancy without side effects for the mother but with transient signs of virilization for the female baby. To find a biochemical marker for the minimal effective dose of androgen derivatives, we measured the plasma levels of C1 C1 INH complexes at different doses of stanozolol in four patients with hereditary angioedema. We found that these complexes, elevated before treatment, promptly reverted to normal values during androgen therapy and remained normal with any reduction of the dose of the drug as long as the patient remained symptom free. Therefore, the measurement of C1 C1 INH complexes appears to reflect the activity of the disease and not the amount of androgen that is administered. (J ALLERGY CLIN IMMUNOL 1991;87:768-73.)

HAE is caused by the deficiency of the C1 INH¹ and is inherited as an autosomal dominant trait. C1 INH deficiency leads to hyperactivation of the classic complement pathway with reductions of plasma C4 and C2 and increase in complexes formed by C1 INH with activated C1.² The defect is highly heterogeneous. Two phenotypic variants were originally described.^{3, 4} It is now known that type I HAE (low antigenic and functional C1 INH levels) may be due to a wide range of gene mutations,⁵⁻⁷ leading to either lack of transcription of messenger RNA or to tran-

Abbreviations used

HAE: Hereditary angioedema

C1: First component of the complement system

INH: Inhibitor

scription in abnormal messenger RNAs that are not translated into a stable protein.^{8, 9} In type II HAE (normal or even increased antigenic levels of C1 INH caused by the presence of a nonfunctional protein), different point mutations have been described within or nearby the reactive center,¹⁰⁻¹² resulting in different dysfunctional proteins.

The clinical syndrome^{13, 14} is characterized by episodic swellings of the extremities, face, bowel wall, and upper airways. Prophylaxis of attacks is successfully accomplished by the use of attenuated androgens (i.e., danazol and stanozolol) alkylated in position 17- α .¹⁵⁻¹⁷ These anabolic steroids increase the plasma levels of C1 INH as well as of several proteins synthesized by the liver.¹⁸ For this reason they have been proposed for the use in other inherited and acquired

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diseases,¹⁹⁻²⁵ although the results were not always as convincing as in HAE. The main concern about HAE treatment with androgen derivatives is that long-term use of these drugs has been associated with an increased risk of hepatocellular and vascular neoplasia.^{26, 27} Such effect depends both on the dose and the duration of the treatment.

Although C1 INH levels approach normal values when patients with HAE are treated with large doses of androgen derivatives, these doses are not necessary for the suppression of attacks. In fact, the therapeutic dose in HAE is not based on a significant increase of C1 INH plasma levels, and for this reason the minimal effective dose is presently established on clinical criteria.²⁸

Because of their toxicity, it is mandatory, in this potentially lifelong lasting treatment, to keep the dose of androgen-derivatives as low as possible. So far, no cases of hepatic neoplasia have been reported in patients with HAE receiving long-term treatment with androgen derivatives.²⁸⁻³⁰ However, the risk-benefit balance of androgen therapy in HAE remains problematic, and a satisfactory biochemical marker of the minimal effective dose would be an advisable aid.

In this article, we report our experience on the long-term (up to 13 years) treatment of HAE with anabolic steroids. Moreover, by measuring the plasma levels of the complexes formed by C1 INH with activated C1, on a pilot group of patients, we evaluated this parameter as a possible biochemical marker for the minimal effective dose of androgen derivative.

MATERIAL AND METHODS

Patients, blood sampling, and storage

One hundred ninety-one patients with HAE from 67 families have been followed. The diagnosis was based on the clinical history and on the low plasma levels of C1 INH function and C4 antigen. Seven families had type II HAE. All patients were white, and their age ranged between 3 and 80 years. Treatment with attenuated androgens was proposed to the adult patients with HAE who suffered from one or more severe attacks a month. In our case list, 41% met this requirement, and 32% (61 patients) started the treatment. Twenty patients were male and 41 were female. Thirty-one patients were treated with stanozolol, 20 with danazol, and 10 patients, in subsequent periods, with both danazol and stanozolol. The treatment lasted for less than 1 year in 12 patients, between 1 and 5 years in 20, between 5 and 10 years in 14, and for more than 10 years in 10 patients. Routine clinical and laboratory controls, especially focused on liver damage, were performed at our clinic or by the family doctor every 3 months for the first year of treatment and yearly thereafter. Plasma for complement tests was drawn into vacutainer tubes (Becton Dickinson, Plymouth, England) containing sodium citrate or ethylenediamine-

tetraacetic acid-polybrene, immediately centrifuged, and stored in polystyrene tubes at -70°C until plasma was tested.

Blood samples from 20 normal subjects, obtained in a similar way, served as controls.

Methods

Functional C1 INH was measured by chromogenic assay with the reagent kit of Immuno (Vienna, Austria). This method is based on the measurement of chromogenic groups released from pyroglutamyl-glycyl-arginin para-nitroaniline (KabiVitrum AB, Stockholm, Sweden), which is specifically split by C1s.

Radial immunodiffusion for C1 INH and C4 was performed according to the method of Mancini et al.³¹ with NOR-Partigen plates (Behringwerke AG, Marburg, Germany).

C1 C1 INH complexes were measured as described.^{32, 33} Briefly, monoclonal antibody, KOK 12, which binds to both complexed C1 INH and cleaved C1 INH, but not to native C1 INH, was coupled to Sepharose beads and incubated with plasma samples. After Sepharose beads were washed with saline, bound complexes were detected by subsequent incubation with ^{125}I -labeled polyclonal anti-C1 antibody. After another washing procedure, Sepharose-bound radioactivity was measured. As standards we used serial dilutions of serum in which a maximal amount of C1 C1 INH complexes was generated by incubation with heat-aggregated human IgG.

Statistical analysis

The differences between groups of patients were evaluated by the nonparametric tests of Wilcoxon and Mann-Whitney U with results expressed as median and range. The evaluation of responses to therapy at various dosages was performed by the paired Student's *t* test with results expressed as mean $\pm$ SD. Correlation between parameters was assessed by linear regression analysis. A *p* value of <0.05 was considered to represent a significant difference or correlation.

RESULTS

Five patients were not available for required routine follow-up clinical and laboratory control evaluations and were thus excluded from the study.

All patients, except two women, reported the complete disappearance of symptoms while they were receiving androgen derivatives. Administration of stanozolol, 4 mg/day, was stopped after 1 year in one patient who still occasionally experienced swellings because of signs of hepatic cell necrosis. After the drug was stopped, HAE symptoms worsened, but liver tests normalized and stanozolol was not resumed. The second patient has been treated for 4 years with various dosages of stanozolol (up to 10 mg/day) and danazol (up to 600 mg/day). Regardless of the amount

TABLE I. Frequency of minimal effective doses of stanozolol and danazol in 56 patients with HAE (10 patients experienced both treatments in different periods)

	Patients (%)
Stanozolol (mg/day)	
1	37
2	56
3	5
4	2
Danazol (mg/day)	
50	20
100	40
200	20
300	20

of the dose, she always complained of a sensation of initial swelling, although her plasma C1 INH function rose from 15% to 57% of normal values, whereas C4 levels normalized. After the drug was stopped, symptoms did not change, and no specific treatment for HAE prophylaxis was started again.

The minimal effective doses for danazol and stanozolol, that is, the dose that caused a complete disappearance of attacks, is reported in Table I. No differences in efficacy or side effects were detected between danazol or stanozolol.

Most of the fertile women (80%) complained of irregularities in menstruation. A slight breast hypotrophy was detected in one 25-year-old woman after 4 years of treatment with stanozolol, 2 mg/day. Initial gain in body weight was reported by 24% of the patients, but weight gain could always be controlled by diet. In two patients, one male and one female, we registered polyglobulia with hematocrit values of 54% and 51%, respectively.

Liver laboratory abnormalities on routine control evaluations were detected only in the patient mentioned above. Androgen treatment was administered to two patients suffering from chronic hepatitis because of the severity of HAE symptoms. No significant changes in liver parameters were detected.

In one woman, androgens were administered from the twenty-sixth to the thirty-fifth week of pregnancy, starting with 400 mg/day of danazol, continuing with 200 mg/day, and ending with 600 mg/day for the last week. An elective cesarean section was made at that time. Surgery and puerperium were uneventful. The body weight of the newborn female infant was 2870 gm with an Apgar score of 10/10. She revealed a transient vulvar edema and a first-degree clitorimegaly. Hormonal assays for thyroid and hypophysis

TABLE II. Complement parameters in two groups of patients with HAE*

	Basal (N = 152)	Treatment (N = 21)	Normal range
C1 INH Ag %†	16 (0-50)	24‡ (9-35)	63-137
C1 INH function %†	16 (0-42)	20§ (16-44)	69-119
C4 Ag %†	19 (0-73)	32‡ (8-77)	67-130

Values are expressed as median and (range).

*Basal conditions (N = 152); treatment with minimal effective dose of anabolic steroids (N = 21).

†Percentage of normal human pooled plasma.

Significance versus basal (Wilcoxon and Mann-Whitney U tests):

‡ $p = 0.0019$.

§ $p = 0.0007$.

|| $p = 0.0065$.

function elicited values in the normal neonatal ranges.³⁴

Mean values of C1 INH antigen and function and of C4 antigen during androgen treatment, at the minimal effective dose, were slightly higher than values observed in patients in basal conditions (Table II).

In four patients, the plasma levels of C1 INH, C4, and C1 C1 INH complexes were measured before and during stanozolol treatment (Table III). The drug was started at a large dose (4 to 6 mg/day, depending on the patient's body weight) and then tapered every 3 weeks to the minimal effective dose, but never <1 mg/day. At different doses of anabolic steroid, a close correlation between the levels of C1 INH and C4 was present ($r = 0.93$; $p < 0.001$), whereas the correlation between C1 INH and C1 C1 INH complexes was less close ($r = 0.594$; $p < 0.05$). In fact, levels of these complexes normalized during androgen treatment and were not dependent on tapering the dose, as long as the patients were symptom free. Only one patient became symptomatic receiving 1 mg/day, and C1 C1 INH complexes were 7.6 U/ml with this dose.

DISCUSSION

Our experience in long-term treatment of HAE with the androgen derivatives, danazol and stanozolol, is largely positive. Because of lack of differences between the two drugs, stanozolol was preferred for its lower cost. We treated more than 50 patients, and these drugs partially failed only in two patients. In one of these patients, there were signs of hepatic cell necrosis that prevented the use of larger doses that may have been effective. The other patient had am-

TABLE III. Complement parameters in four patients with HAE at different doses of stanozolol (high, 4 to 6 mg/day; medium, 2 to 3 mg/day; low, 1 to 2 mg/day)

	Pretreatment (N = 4)	High dose (N = 4)	Medium dose (N = 4)	Low dose (N = 4)
C1 INH Ag %*	17 ± 2	48 ± 20†	36 ± 9†	27 ± 8
C1 INH function %*	11 ± 2	31 ± 6†	23 ± 4†	16 ± 4†
C4 Ag %*	20 ± 7	79 ± 27†	65 ± 14†	49 ± 16
C1 C1 INH U/ml	9.1 ± 1.8	2.4 ± 0.5‡	1.7 ± 0.7‡	2.9 ± 1.8†

Values are expressed as Mean ± SD.

Normal values for C1 C1 INH complexes, 0.4 to 3.0 U/ml.

C1 C1 INH complexes in patients with HAE in remission, 0.8 to 7.7 U/ml.

Normal values for the other parameters are reported in Table II.

*Percentage of normal human pooled plasma.

Significance versus basal (paired Student *t* test):

†*p* < 0.05.

‡*p* < 0.01.

biguous symptoms that always remained subjective and were present despite significant rises in C1 INH and C4. Therefore, we think that in this patient the symptoms were not related to angioedema and not due to ineffectiveness of the drug.

The minimal effective doses in our patients are significantly smaller compared to doses reported in a previous study²⁸ on a similar case list. With these dosages, serious side effects were never registered, even in those patients continuously treated for 13 years. Two patients experienced mild polyglobulia, an effect that has been already reported in long-term treatment with anabolic steroids.³⁵

Most patients that we treated were women, and none of the women had signs of virilization. Only in one patient was there a slight mammary hypotrophy. Minor irregularities in menstruations were frequently reported, but only large daily dosages (4 mg of stanozolol and 400 mg of danazol), administered as initial treatment, resulted in amenorrhea. The patient treated with danazol during pregnancy did not register any adverse effects, but her baby demonstrated mild signs of virilization. These results confirm that danazol, even when it is administered at low doses and late in the pregnancy, that is, after sexual differentiation of the fetus, can interfere with normal female external genital phenotype. Therefore, a careful evaluation appears to be appropriate when attenuated androgens are proposed for pregnant women.³⁶

Increase in body weight, acne, masculinization, and sensation of puffy skin were reported as a dose-related reaction in a previous study.²⁸ These effects were occasionally reported by our patients as a minor complaint at the beginning of the treatment when the dosage was larger, but not at the minimal effective dose.

The major concern of hepatoma induction by long-

term treatment with androgen derivatives^{26, 27} is not confirmed by our results. The present study suggests that the doses of androgen derivatives used in HAE appear to spare the liver despite the length of therapy, even in the patients affected with chronic non-A, non-B hepatitis.

The median levels of plasma C1 INH and C4 in our patients, at the minimal effective dose, demonstrated a statistically significant increase in both proteins compared to the levels determined in patients in basal conditions. However, these parameters, during and outside treatment, largely overlapped, and for this reason, they cannot be used to establish the minimal effective dose. Therefore, we studied four patients with HAE receiving different doses of stanozolol to evaluate the advantage of measuring the plasma levels of C1 C1 INH complexes over the usual complement parameters as a marker of treatment effectiveness. It has been demonstrated¹⁶ that during androgen treatment the levels of C4 were directly related to the concentrations of C1 INH antigen up to a concentration of about half the C1 INH found in normal plasma; at this point, the C4 concentrations usually are in the normal range and no longer correlate well with C1 INH concentration. Our four patients demonstrated a similar trend with a dose-related increase in C1 INH and C4, whereas C1 C1 INH complexes behaved differently. We previously reported that patients with HAE in remission have higher levels of C1 C1 INH complexes compared to normal subjects because of C1 INH consumption via C1 activation.² In the four patients of the present study, plasma levels of the complexes before treatment were above the levels detected in patients with HAE in remission. We ascribed this to the fact that the four patients were in a highly symptomatic period,³⁷ and for this reason, they were

starting with the long-term prophylaxis. Androgen treatment induced a drop of the complexes to normal levels that did not change on reduction of the dose as long as the patient remained symptom free. In one patient, who became symptomatic when stanozolol was tapered to 1 mg/day, C1 C1 INH complexes rose to pretreatment levels. Therefore, the measurement of C1 C1 INH complexes (as an index of C1 INH consumption) appears to reflect the activity of the disease and not the amount of androgen that is administered. Measurement in a larger number of patients will be necessary to confirm this statement. At the present time, C1 C1 INH complexes appear to be a promising biochemical marker of the minimal effective dose of attenuated androgen in long-term treatment of HAE.

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Altered T cell subpopulations and lymphocytes expressing natural killer cell phenotypes in patients with progressive systemic sclerosis

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Scleroderma (progressive systemic sclerosis [PSS]) is known to be associated with abnormal T cell immunoregulation. In the present study, we evaluated lymphocyte phenotypes in patients with PSS and normal control subjects by flow cytometry and monoclonal antibodies for total T (CD3), T suppressor (CD8), T helper (CD4), T helper-inducer (CDw29), T suppressor-inducer (CD45R), human leukocyte antigen, DR+B (CD19), DR+T, and natural killer subsets, HNK-1 (CD57) and NKH-1 (CD56) cells. Patients with PSS compared to normal subjects had significantly lower percentages of CD3+ (p < 0.005) and CD8+ (p < 0.05) (similar to several patients with rheumatoid arthritis also evaluated), as well as CD45R (p < 0.05), T+DR+ (p < 0.05), and NKH-1 (CD56) (p < 0.0005) cells. Patients with PSS with late-limited or generalized disease had lower percentages of CD8+, CD19, NKH-1+, and CDw29, but higher percentages of CD4+, HNK-1, and CD45R cells compared to patients with early stage disease, but these results were not statistically significant. These unique alterations in patients with PSS may prove to be useful in monitoring the stage of disease activity for therapy and further define immunologic defects. (J ALLERGY CLIN IMMUNOL 1991;87:773-9.)

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Abbreviations used

Ia:	Immune response gene-associated
PSS:	Progressive systemic sclerosis
NK:	Natural killer
RA:	Rheumatoid arthritis
CREST:	Calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, and telangiectasia
HBSS:	Hanks' balanced salt solution
FITC:	Fluorescein isothiocyanate
MAb:	Monoclonal antibody
PBMC:	Peripheral blood mononuclear cell