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ORIGINAL ARTICLE

Long-term efficacy of danazol treatment in hereditary angioedema

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ABSTRACT

Background No systematic study has been published yet on the long-term efficacy of attenuated androgens in hereditary angioedema (HAE). Our aim was to conduct a follow-up study in two (German and Hungarian) cohorts of HAE patients (45 and 39 patients, respectively) undergoing uninterrupted treatment for 6 years with similar (starting dose 128 ± 78 mg per day and 136 ± 70 mg per day, respectively) and constant doses of danazol.

Design The frequencies of subcutaneous, abdominal and laryngeal attacks were recorded each year.

Results The annual frequency of all the three types of attacks was significantly lower during the first year of danazol treatment, compared to the last year before baseline. During subsequent years in Hungarian patients, the frequency of both subcutaneous and abdominal attacks – but not that of laryngeal attacks – increased significantly. In the case of abdominal attacks, a significant increase in the attack frequency was observed only in female patients. In the German cohort, by contrast, no change in the frequency of either type of attack was found during the 6-year study period.

Conclusions The differences observed between these cohorts cannot be related to drug dose, the age or gender distribution of subjects or the age at the onset of symptoms or the length of diagnostic delay in the patients. There were, however, marked differences in the baseline pattern of attacks: significantly – 3 times – more abdominal attacks were recorded in German patients. Further studies are necessary to clarify the mechanism of these findings.

Keywords attenuated androgen, hereditary angioedema, long-term prophylaxis.

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Introduction

Hereditary angioedema (HAE), because of C1-inhibitor deficiency, is clinically characterized by recurrent skin swelling, paroxysms of abdominal pain and life-threatening upper airway obstruction. The attacks of the disease are treated with C1-inhibitor concentrate and recently by administering a bradykinin receptor inhibitor. The majority of HAE patients require long-term prophylaxis to decrease the frequency and severity of attacks. The drugs of choice for long-term prophylaxis are attenuated androgens, of which 17-α-alkylated androgens (such as danazol, stanozolol and oxandrolone), predominantly danazol, are used recently [1].

Danazol was first developed in 1963 as a synthetic analogue of ethinyltestosterone. Attenuated androgens had been introduced in the therapy of HAE during the seventies [2] and were first evaluated in the early eighties [3–5]. The efficacy of this type of treatment was demonstrated by several articles and reviews, in several thousand patients altogether [6–10]. Danazol and the other attenuated androgens are well tolerated by most patients; however, long-term prophylaxis with these drugs may be accompanied by serious side effects, which lead to the discontinuation of treatment in a significant proportion of patients [11]. Danazol is not recommended for children, and it is contraindicated also in pregnant women. In adults, danazol can cause weight gain, microscopic hematuria, hepatic side effects, liver tumours, anxiety, altered libido, alopecia and hypertension [1,11–14]. Danazol treatment may lead to the formation of liver adenomas [15].

According to the recent study by Kreuz *et al.*, [16], the most common adverse events of danazol treatment include depression, headache, increased body weight, amenorrhoea, virilization and hypertension. Liver adenoma was identified in two patients (9%). Earlier, we described the atherogenic lipid profile

in HAE patients treated with Danazol [17], which, however, was not associated with manifest atherosclerosis, i.e. an increased carotid intimal-medial thickness [18,19].

In contrast to the abundance of literature data on the side effects of therapy with attenuated androgens, meagre information is available on the long-term efficacy of these drugs in HAE. As the follow-up data of HAE patients are regularly collected in both German and Hungarian institutions, we decided to study whether prophylactic danazol therapy retains its efficacy during a long-term follow-up of 6 years. Forty-five German and 39 Hungarian HAE patients receiving uninterrupted treatment with almost constant doses of danazol over a 6-year period were followed up. The frequency of subcutaneous, abdominal and laryngeal attacks was registered for each year, and eventual changes of attack frequencies were evaluated. Additionally, the frequencies of the three types of attack were summarized for the year before the start of danazol therapy and were compared to those measured during the first year of treatment.

Materials and methods

Patients

Germany. Forty-five patients (23 men, 22 women) with HAE (type 1: 43, type 2: 2) managed by the Department of Dermatology of Johannes Gutenberg University (Mainz, Germany) were included in the study. At the time of the start of danazol treatment, these patients were 33·7 ± 12·7 years old – 4 patients (9%) (3 men, 1 women) were younger than 18 years of age. The initial dose of danazol was 136 ± 70 mg per day. All patients had been receiving danazol without interruption since at least 6 years.

Hungary. Thirty-nine patients (19 men, 20 women) with HAE (type 1: 35, type 2: 4) followed up at the 3rd Department of Internal Medicine of Semmelweis University (Budapest, Hungary) were enrolled. Upon initiating treatment with danazol, these patients were 31·8 ± 13·1 years old; 8 patients (21%) (7 men, 1 women) were under the age of 18 years. The starting dose of Danazol was 128 ± 78 mg per day. Similar to the German subset of patients, all these subjects had been receiving danazol without interruption since at least 6 years.

Recording clinical manifestations in the patients' diary

Of the data recorded by the patients themselves in their diaries, the following were used:

- time of the onset of individual attacks;
- the localization of symptoms and
- the treatment potentially administered to relieve the attack.

The manifestations recorded in the patient diaries were transferred into the database by the attending physician at each visit (that is, at least once a year). The same diaries adapted (only) from the same source (Patient Diary of the CSL Behring), translated to Hungarian and German, respectively, were used in the two centres. The patients were given the same instructions, and the clinicians evaluated similarly the patients' diaries in the two centres.

The study protocol was approved by the Research Ethics Committee, and the study was conducted in accordance with the Declaration of Helsinki.

Reporting of the study conforms to STROBE along with references to STROBE and the broader EQUATOR guidelines (Simera *et al.* January 2010 issue of EJCI).

Statistical analysis

The nonparametric version of the repeated measures ANOVA (Friedman's test), supplemented with Dunn's *post hoc* test or the Wilcoxon test, was applied for evaluating the changes over the whole follow-up period, as well as for comparing attack frequencies measured at baseline and during the first year or in the first and sixth years of therapy. The differences between the frequencies recorded in the German and in the Hungarian databases were evaluated by the Mann–Whitney test. All tests were two tailed. Statistical analysis was performed using the GraphPad Prism 3.0 (GraphPad Software Inc, San Diego, CA, USA, http://www.graphpad.com) and SPSS 13.0 (SPSS Inc., Chicago, IL, USA) software.

Results

Differences between the baseline frequencies of the different types of attacks as well the age at onset of the symptoms and at diagnosis in Hungarian and in German HAE patients

The total number of subcutaneous, abdominal and laryngeal attacks recorded during the year before the start of danazol treatment in 39 Hungarian and in 45 German HAE patients were compared to each other (Table 1). There was no significant difference in the occurrence of subcutaneous attacks between the Hungarian and German patients. By contrast, significantly – 3 times – more abdominal attacks were observed in German patients, whereas the frequency of laryngeal attacks was significantly higher among Hungarian patients. The baseline proportion of subcutaneous/abdominal attacks was 1·2 and 2·6 in the German and in the Hungarian cohort, respectively. On the other hand, laryngeal attacks were relatively infrequent (corresponding to 2% of subcutaneous attacks among German patients, when compared to 11% in the Hungarian cohort). No significant differences in the age at the onset of symptoms, age at the establishment of

Table 1 Baseline differences between the 45 German and 39 Hungarian patients studied

	Germany (45 patients)	Hungary (39 patients)	*P* value (Mann–Whitney test)
Subcutaneous attacks per year, mean ± SD	18·58 ± 18·32	13·22 ± 10·21	0·659
Abdominal attacks per year, mean ± SD	15·73 ± 13·52	5·18 ± 6·32	<0·001
Laryngeal attacks per year, mean ± SD	0·29 ± 0·59	1·40 ± 2·19	<0·001
Age at the first symptoms, years, mean ± SD	12·2 ± 9·3	9·3 ± 6·2	0·2108
Age at establishment of diagnosis, years, mean ± SD	31·5 ± 12·5	30·1 ± 13·8	0·3843
Diagnostic delay years, mean ± SD	19·3 ± 11·5	20·8 ± 15·7	0·8682

diagnosis or the length of diagnostic delay were found between the two cohorts (Table 1).

The initial efficacy of danazol treatment in the two patient cohorts

First, the total numbers of subcutaneous, abdominal and laryngeal attacks recorded during the year before the start of danazol treatment as well as in the initial year of therapy were compared to each other in the 39 Hungarian HAE patients. We considered year 1 the calendar year in which the patient got his or her first danazol treatment and the year before the start of danazol treatment was the calendar year which preceded it. The number of attacks in this year was registered according to the diaries or if the patient's treatment began just after the establishment of the diagnosis according to his or her memories (Fig. 1). The introduction of danazol treatment led to a significant decrease in the frequency of all the three types of attacks.

The results found in the 45 German patients are shown in Fig. 2. Similar to the other cohort, significantly less subcutaneous, abdominal and laryngeal attacks occurred during the first year of treatment, than in the preceding year (Fig. 2.).

The changes in the annual frequencies of the individual types of attacks during 6 years of uninterrupted danazol treatment were markedly different in the Hungarian and German cohorts

Thirty-nine Hungarian patients were treated with a constant dose (146 ± 84 mg per day and 135 ± 47 mg per day, respectively, for year 1 and 6 of the treatment, $P = 0{\cdot}7869$) of danazol for 6 years (Fig. 3.). Changes in the frequencies of the three types of attacks were evaluated by the nonparametric Friedman's test, followed by Dunn's *post hoc* test for paired comparisons. There was a significant increase in the frequency of both subcutaneous and abdominal attacks, but not in that of

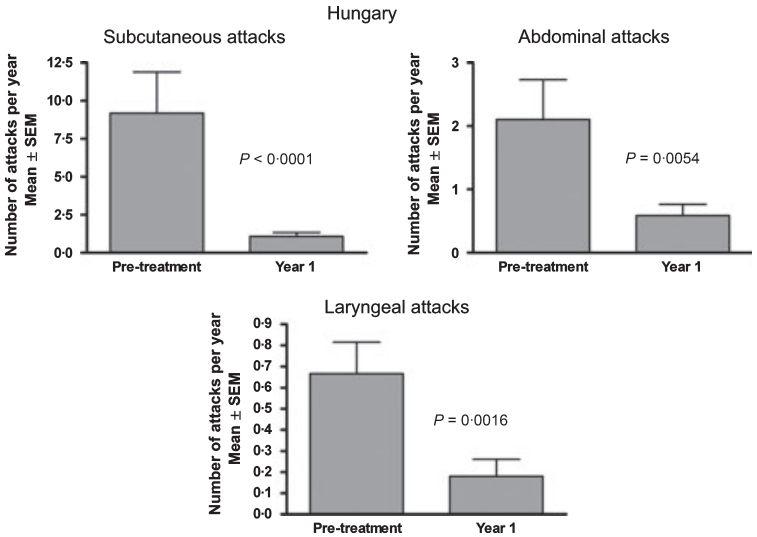

Figure 1 Changes in the annual frequencies (mean ± SEM) of subcutaneous, abdominal and laryngeal attacks in the year before the start and during the first year of danazol treatment in 39 Hungarian patients with hereditary angioedema. *P* values for Wilcoxon test are indicated.

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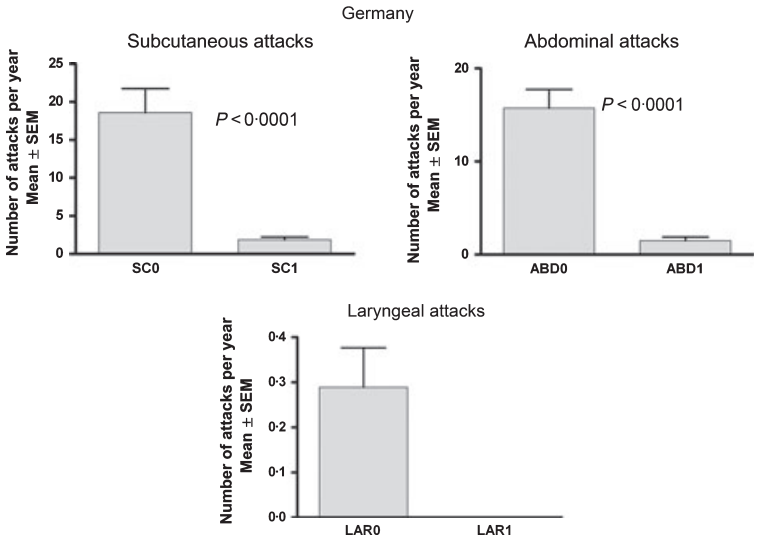

Figure 2 Changes in the annual frequencies (mean ± SEM) of subcutaneous, abdominal and laryngeal attacks in the year before the start and during the first year of danazol treatment in 45 German patients with hereditary angioedema. *P* values for Wilcoxon test are indicated.

laryngeal attacks. The annual frequency of subcutaneous attacks was significantly ($P = 0{\cdot}002$) higher in the sixth year (3·56 ± 4·41, mean ± SD), than during the first year (1·08 ± 1·55).

Next, we checked the changes in attack frequencies over the 6 years in male and in female patients. Comparing attack frequencies from the first and the sixth year revealed remarkable gender-related differences (Table 2). While the frequency of subcutaneous attacks increased in both sexes, abdominal attacks occurred significantly more frequently in the sixth, than in the first year only among female patients ($P = 0{\cdot}007$), whereas no significant changes occurred in male patients (0·530). The p values (Friedman's test) were 0·0008 and 0·8702 for female and male patients, respectively.

Quite different results were obtained in the 45 German HAE patients treated with a constant dose (136 ± 70 mg per day and 139 ± 84 mg per day during the first and sixth year of treatment, respectively, $P = 0{\cdot}9375$) of danazol (Fig. 4). The frequencies of the three types of attacks remained unchanged during the 6 years of danazol treatment. No changes were found even if male and female patients were evaluated separately.

As the number of young (<18 years old) patients was greater in the German cohort, we repeated the evaluation by restricting the analysis to adult (≥18 years old) patients only; however, this revealed the same differences between the two cohorts. Specifically, the frequency of subcutaneous attacks was significantly ($P = 0{\cdot}008$) higher during year 6, than in year 1 of danazol therapy among adult Hungarian patients, but no significant difference ($P = 0{\cdot}475$) was found in the German cohort.

We have also compared the number of attacks which had to be treated with C1-inhibitor concentrate in the two cohorts and found that the concentrate use was much higher ($P < 0{\cdot}0001$) in the Hungarian than in the German cohort, although no consequent changes in time in the concentrate use were found in either group of patients.

Discussion

This two centre, comparative study of the rare disease, HAE, conducted in the sole Hungarian and in a large German centre on the long-term efficacy of treatment with the attenuated androgen danazol yielded interesting and contrasting findings. During 6 years of uninterrupted prophylaxis with almost constant doses of danazol, the efficacy of the drug was found unchanged in German patients. In Hungary, by contrast, the frequency of subcutaneous and abdominal attacks had increased steadily, and it was significantly higher during the last year, than in the first year of treatment. Interestingly enough, the rise of abdominal attacks occurred only in female patients, whereas the frequency of subcutaneous attacks increased in both women and men.

This difference was not related to the intensity of danazol treatment, as its daily dose was almost the same in the two cohorts. The proportions of male and female patients, the mean age of the subjects as well as the age at the first symptoms and the length of diagnostic delay were also comparable in the German and in the Hungarian subpopulations. The proportion of young (<18 years old) patients was higher (20% vs. 9%) in the

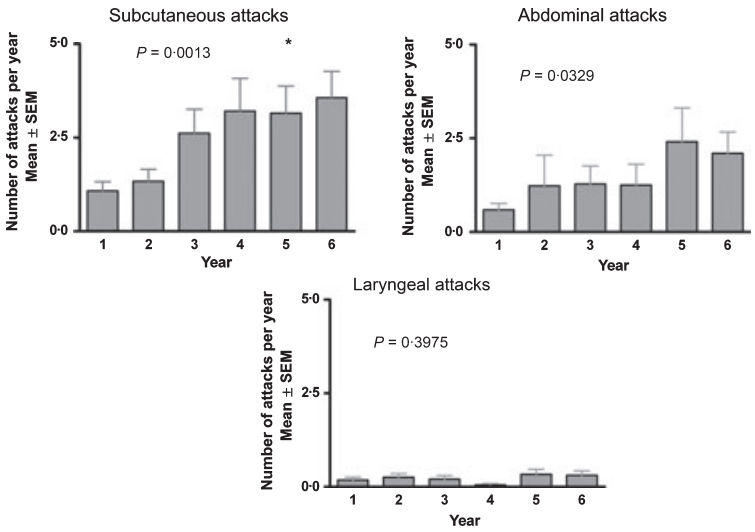

Figure 3 Changes in the annual frequencies (mean ± SEM) of subcutaneous, abdominal and laryngeal attacks during the 6-year follow-up period in 45 Hungarian patients undergoing uninterrupted danazol therapy for hereditary angioedema. *P* values for the Friedman's test and Dunn's *post hoc* test (**P* < 0·05) are indicated.

Table 2 Differences between annual attack frequencies measured in the first (Y1) and sixth (Y6) year of danazol treatment in Hungarian male and female patients with hereditary angioedema

	Subcutaneous attacks per year, mean ± SD			**Abdominal attacks per year, mean ± SD**			**Laryngeal attacks per year, mean ± SD**		
	Y1	**Y6**	***P****	**Y1**	**Y6**	***P****	**Y1**	**Y6**	***P****
Males (*n* = 19)	1·14 ± 1·57	3·32 ± 4·15	0·04	0·39 ± 0·82	0·89 ± 1·88	0·53	0·16 ± 0·50	0·32 ± 0·95	0·68
Females (*n* = 20)	1·00 ± 1·52	3·60 ± 4·76	0·02	0·80 ± 1·32	3·25 ± 4·35	0·007	0·20 ± 0·52	0·30 ± 0·57	0·48

*Wilcoxon test.

German cohort, than in the Hungarian, but notwithstanding this the difference was the same when only the adult patients were taken into account. Additionally, the initial efficacy of danazol treatment was rather similar in the two centres (Figs 1 and 2).

The only marked difference between the German and the Hungarian cohort was observed in the baseline frequency of abdominal and laryngeal attacks (Table 1). On average, the mean annual number of abdominal attacks during the year preceding the first year of danazol treatment was significantly – that is, three times – higher in the German than in the Hungarian cohort. The baseline proportion of subcutaneous/abdominal attacks was 1·2 in the German and 2·6 in the Hungarian cohort. The data of German patients are in agreement with a previous analysis of 131,110 attacks observed in 223 HAE patients [20]: 50% vs. 48% of the attacks were localized to the skin and to the gut/stomach, respectively. On the other hand, laryngeal attacks were relatively infrequent (again in conformity with the previous analysis [20]), i.e. corresponded to 2% of subcutaneous attacks, compared to the 11% seen in the Hungarian cohort.

These differences suggest that the natural course of the disease was different in the two cohorts, possibly because of genetic differences. It cannot be excluded either that the indication for danazol therapy was based on slightly different criteria in German and in the Hungarian centre. Clearly, further studies are necessary to reveal the causes of the observed differences. Although no similar systematic longitudinal studies have been reported so far, a recent observation indicates that some patients might be at least partially resistant to danazol treatment – such as those who discontinue long-term prophylaxis with danazol because of lack of efficacy [16].

The next issue to be discussed is the means whereby danazol lost its attack-preventing efficacy in Hungarian HAE patients. Considering that the exact mechanism of the clinical effect of attenuated androgens is not known, this question cannot be answered yet. A widely accepted, tentative mechanism of attack-prophylaxis by danazol is the enhanced synthesis of

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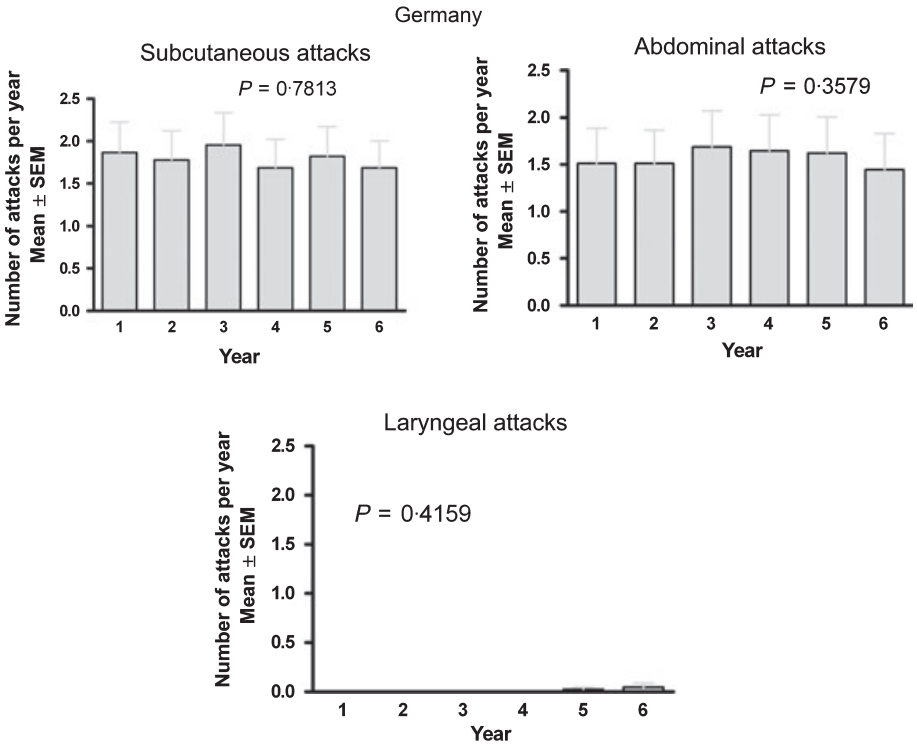

Figure 4 Changes in the annual frequencies (mean ± SEM) of subcutaneous, abdominal and laryngeal attacks during the 6-year follow-up period in 45 German patients receiving uninterrupted danazol therapy for hereditary angioedema. *P* values for the Friedman's test are indicated.

proteins, including C4 and C1-INH [21]. *In vitro*, androgens indeed increase the synthesis of C1-INH in monocytic and hepatoma cell lines [22], albeit a direct effect of androgens on C1-INH synthesis has not been demonstrated convincingly [23,24]. Furthermore, the symptomatic benefit is generally achieved at doses lower than those required to effect a significant change in complement levels [25,26]. Several other mechanisms have also been suggested. For example, testosterone can inhibit bradykinin-induced intracellular calcium kinetics in cultured rat aortic endothelial cells [27]. Further, testosterone inhibits bradykinin-induced relaxation of coronary artery rings [28]. These diverse effects illustrate how testosterone might inhibit oedematous symptoms in patients with HAE. Recently, it was demonstrated by Thon *et al.* [29] that in patients with HAE, danazol strongly increased testosterone in relation to precursors and downstream oestrogen. Danazol treatment in HAE may mitigate oedema by increasing the levels of highly active androgen testosterone and thereby interfering with several pathophysiological pathways of local oedema formation [29]. Our study, exploring the differences between the long-term efficacy of danazol treatment of Hungarian and German (and possibly other) cohorts, may cast some light on the mechanism of the therapeutic effect of attenuated androgens in HAE. On the other hand, the current modality of prophylactic treatment exposes HAE patients to a significant risk of adverse effects, and while the efficacy of prophylaxis is generally good, it is far from perfect [26]. These findings indicate that attenuated androgens may loose their efficacy over time. Therefore, seeking new therapeutic modalities – not only for the treatment of attacks but also for long-term prophylaxis – seems justified.

Disclosure

Authors disclose any financial relationship with a biotechnology and/or pharmaceutical manufacturer that would have an interest in the subject matter or materials discussed in the submitted manuscript.

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