Danazol-induced Augmentation of Serum α1-Antitrypsin Levels in Individuals with Marked Deficiency of this Antiprotease

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ABSTRACT Individuals with serum α1-antitrypsin levels below 80 mg/dl are clearly at risk for the development of accelerated panacinar emphysema. One possible approach to the therapy of this disorder would be to raise serum levels of this major antiprotease to establish protease-antiprotease homeostasis within the lung parenchyma. Because danazol, an impeded androgen, elevates levels of C1 inhibitor in patients deficient of that serum antiprotease, we hypothesized that this agent might also increase α1-antitrypsin levels in patients with α1-antitrypsin deficiency. To evaluate this concept, seven patients with severe emphysema associated with α1-antitrypsin deficiency (six PiZ and 1 $M_{Duarte}Z$) and one asymptomatic individual (PiSZ) received 600 mg of danazol daily for 30 d. Five of the six PiZ patients responded to danazol therapy with significant increases in serum α1-antitrypsin levels (mean increase of 37%; $P < 0.03$). The two individuals who were heterozygous for the Z protein increased their serum levels by 85% ($PiM_{Duarte}Z$) and 87% (PiSZ), respectively. These increases in serum α1-antitrypsin antigen were accompanied by commensurate increases in serum trypsin inhibition. Crossed immunoelectrophoresis showed no alterations of the microheterogeneity of the α1-antitrypsin or the presence of protease-antiprotease complexes in serum during danazol therapy. These data demonstrate that serum α1-antitrypsin levels can be augmented by danazol therapy in PiZ individuals as well as those heterozygotes with severe deficiency of α1-antitrypsin. The clinical relevance of these increases in serum α1-antitrypsin remains speculative, but these findings suggest that danazol may provide a means of improving the protease-antiprotease balance in these individuals and thus impede the progression of their lung disease.

INTRODUCTION

α1-Antitrypsin is a major serum antiprotease with more than 20 known phenotypes (1–4). Individuals homozygous for the Z variant (phenotype PiZ) or heterozygous for the Z and M_{Duarte} variants (Pi $M_{Duarte}Z$) and Z and S variants (Pi SZ) are distinguished by marked deficiency of serum α1-antitrypsin levels and a predisposition to severe panacinar emphysema (5–7).

The normal α1-antitrypsin protein (M) is synthesized by the hepatocyte and released into the circulation at a rate of 38 mg/kg-d (8). Current evidence suggests that the serum α1-antitrypsin deficiency in individuals with phenotypes PiZ, $M_{Duarte}Z$, and SZ seems to result from an inability of the hepatocyte to transport the variant protein(s) to the extracellular space (3). It is likely that in each variant, this abnormality derives from a base substitution in the structural gene for α1-antitrypsin, resulting in an amino acid substitution that alters the conformation of the protein (9–11) and ultimately its secretion from the hepatocyte.

α1-Antitrypsin is the major serum antiprotease capable of inhibiting neutrophil elastase (12). Current concepts of the pathogenesis of the emphysema associated with marked α1-antitrypsin deficiency hold that the consequence of low levels of circulating α1-antitrypsin is progressive, unimpeded neutrophil elastase-mediated destruction of lung parenchyma (2–4). According to this concept, individuals with α1-antitrypsin phenotypes PiZ, $M_{Duarte}Z$, and SZ are at risk for the development of emphysema owing to this protease-antiprotease imbalance in lung. Because the

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variant α1-antitrypsin phenotypes are as effective as the M protein as antiproteases (3), it follows therefore, that if serum α1-antitrypsin levels could be augmented in α1-antitrypsin deficient individuals, protease-antiprotease homeostasis would be established with resultant protection of lung structures from protease-induced emphysema.

One therapeutic approach to increasing α1-antitrypsin levels in PiZ, $M_{Duarte}Z$, and SZ individuals is to induce the hepatocyte to increase its output of α1-antitrypsin. Theoretically, optimal therapy would use an agent that specifically stimulates the hepatocyte to increase its output of α1-antitrypsin. Although such an agent is unknown, it is recognized that anabolic steroids such as diethylstilbestrol will stimulate hepatocytes to increase the production of various proteins, including α1-antitrypsin (13). Unfortunately, the use of diethylstilbestrol is associated with estrogenic side effects, thus precluding its chronic use in a disorder such as α1-antitrypsin deficiency. Recently, however, it has been shown that a synthetic androgen, danazol (2,3-isoxazol-17 α-ethinyl testosterone), will also induce the hepatocyte to increase production of various proteins without many of the dangers associated with other anabolic steroid agents (14). This, together with the fact that danazol increases serum C1 inhibitor levels in hereditary angioedema (another serum antiprotease deficiency disorder), suggests that danazol might be an effective therapy for severe α1-antitrypsin deficiency (14–16). For these reasons, the present study was designed to assess the capacity of danazol to augment serum α1-antitrypsin levels in individuals with α1-antitrypsin phenotypes PiZ, $M_{Duarte}Z$, and SZ.

METHODS

Patient population. The study population consisted of eight individuals referred to the Pulmonary Branch, National Institutes of Health, with known decreased serum levels of α1-antitrypsin (Table I). Seven had bibasilar lower lobe bullous disease and one had a normal chest roentgenogram. Of the seven patients with bullous disease, six possessed the phenotype PiZ. These homozygous patients included three males and three females with average age of 47±5 yr. This group had experienced pulmonary symptoms for 5±3 yr; none were active cigarette smokers at the time of the study, although four were ex-smokers who had averaged 25±4 pack-yr of cigarette consumption. Pulmonary function testing (17) of the PiZ individuals revealed severe obstruction to airflow and reduced diffusing capacity consistent with the clinical diagnosis of emphysema. One of the seven patients with bullous lung disease was a heterozygote with the $PiM_{Duarte}Z$ phenotype. This individual was a 43-yr-old female. She was an ex-smoker who had consumed 20 pack-yr of cigarettes, and whose pulmonary function tests closely resembled those of the six homozygous patients. The only asymptomatic subject included in the study was a 19-yr-old with phenotype PiSZ, a nonsmoking female whose pulmonary function studies were normal.

α1-Antitrypsin serum levels and α1-antitrypsin phenotyping. Serum levels of α1-antitrypsin in the study patients were measured by a standard radial immunodiffusion method using antibody-containing agarose plates and reference serum standard (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.). Serum levels of α2-macroglobulin and antithrombin III were measured using plates obtained from the same source; serum C1 inhibitor was measured by the same technique. All determinations were performed in duplicate according to the methods described by Mancini (18).

TABLE I
*Clinical and Physiological Description of the Study Population**

α1-Antitrypsin phenotype	Patient	Age	Sex	Duration of symptoms	Smoking history	Serum α1-antitrypsin	Vital‡ capacity	Total lung capacity	RV/TLC§	FEV_1‖	MMEF¶	D_{LCO}**
		yr		*yr*	*pack-yr*	*mg/dl*	*% predicted*		*% observed*		*% predicted*	
PiZ	R.J.	49	M	4	30	15	78	128	51	24	10	36
PiZ	L.E.	37	M	2	20	35	49	112	67	23	10	35
PiZ	M.G.	66	F	5	0	43	111	103	38	51	8	40
PiZ	H.P.	50	F	15	30	27	47	71	54	21	4	26
PiZ	T.W.	34	M	7	20	32	47	95	64	12	4	19
PiZ	E.M.	49	F	2	0	42	51	81	36	29	12	38
		47±5*		5±3		32±4	64±10	98±8	52±5	27±5	8±1	32±3
$PiM_{Duarte}Z$	D.K.	43	F	11	20	20	38	71	48	16	7	36
PiSZ	A.J.	19	F	0	0	85	96	87	18	103	91	77

* Mean±SE of the M are given for the PiZ group.
‡ Methods for the physiologic studies (17).
§ RV/TLC, residual volume/total lung capacity.
‖ FEV_1, forced vital capacity in 1 s.
¶ MMEF, maximum midexpiratory flow rate (25–75% of vital capacity).
** D_{LCO}, diffusing capacity; single breath method.

All data given as mean±SEM; all statistical comparisons are by the two-tailed Student's *t* test.

The eight patients were phenotyped by acid starch gel electrophoresis of their pretreatment serum (19). Six of the eight subjects were homozygous for the Z protein (Pi Z). The seventh was heterozygous for the Z protein and M_{Duarte} protein (Pi M_{Duarte} Z; the Duarte protein displayed an electrophoretic mobility identical to the M protein (7), yet was associated with serum levels that approximated those of the Z protein). The eighth individual was heterozygous for the S and Z proteins.

Drug protocol. Before initiation of the study, each patient was determined to be free of clinical or biochemical evidence of hepatic, renal, or hematologic disease. The participants each received 600 mg of danazol orally per d for 30 consecutive d. Serum was obtained on two occasions before administration of the test drug and thereafter throughout the 30-d study period at intervals that ranged from 3 to 7 d. These sera were kept in liquid N_2 vapor until assayed. Serum transaminase levels, alkaline phosphatase, and bilirubin, were measured at the conclusion of the study to assess any hepatic dysfunction secondary to the use of danazol. Given the short duration of this drug study, no attempt was made to ascertain the influence of this mode of therapy on the severe, chronic pulmonary disease manifested in the study patients.

Serum trypsin inhibitory capacity. To verify the functional integrity of the measured antigenic α1-antitrypsin in serum, base line and 30-d sera were assayed for the capacity to inhibit the enzymatic activity of bovine pancreatic trypsin (Sigma Chemical Co., St. Louis, Mo.) on the synthetic substrate benzoyl-arginine-*p*-nitroanilide as described by Eriksson (20).

Bidirectional antigen-antibody electrophoresis. To evaluate possible alterations in the microheterogeneity of α1-antitrypsin secondary to danazol therapy, pretreatment, and 30-d treatment sera were evaluated using crossed immunoelectrophoretic analysis (21). Isoelectric focusing was performed in polyacrylamide (pH 4–5); for the second dimension, the polyacrylamide strip containing the serum sample was electrophoresed into agarose (pH 8.2) containing monospecific goat antiserum to α1-antitrypsin (Atlantic Antibodies, Westbrook, Maine). To evaluate sera for protease-α1-antitrypsin complexes, crossed immunoelectrophoresis under alkaline conditions using agarose in the first dimension was performed as described by Laurell (22).

RESULTS

The group mean pretreatment α1-antitrypsin levels for the six homozygous PiZ patients was 32 mg/dl. After 30 d of danazol therapy, the group mean level was 44 mg/dl representing an average increase of 37% over pretreatment levels ($P < 0.03$) (Fig. 1). Evaluation of the sera of each of the homozygous PiZ patients demonstrated that, compared with their individual pretreatment levels, five of the six patients had significant increases in their serum α1-antitrypsin levels within 3 wk of therapy. The one PiZ patient who did not respond (M.G.) had a pretreatment level of 43 mg/dl and a posttreatment level of 42 mg/dl. In those patients that responded to danazol, the α1-antitrypsin levels returned to approximately pretreatment levels within 7 d after cessation of therapy (data not shown).

The two heterozygous patients (D.K., phenotype Pi M_{Duarte}Z; A.J., phenotype Pi SZ) both responded to danazol therapy. The Pi M_{Duarte}Z patient went from a serum α1-antitrypsin pretreatment level of 20 mg/dl to a level of 37 mg/dl after 3 wk of therapy. The SZ patient had the largest absolute elevation of α1-antitrypsin levels of all study patients (pretreatment, 85 mg/dl; after 3 wk of therapy, 160 mg/dl).

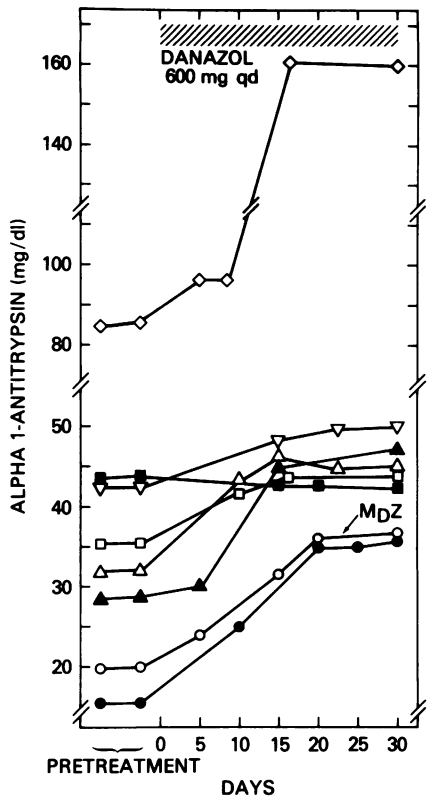

FIGURE 1 Effect of danazol administration on serum α1-antitrypsin levels in the eight study patients (R.J., ●; D.K. ○; H.P., ▲; T.W., △; M.G., ■; L.E., □; E.M., ▽; A.J., ◇). All individuals were type PiZ except D.K. (PiM_DZ) and A.J. (PiSZ).

Crossed antigen-antibody electrophoresis demonstrated that the increment in serum α1-antitrypsin in the patients who responded to danazol was not attributable to protease-α1-antitrypsin complexes (data not shown). In addition, comparison of isoelectric focusing-crossed immunoelectrophoresis patterns in sera obtained before and during danazol therapy revealed no apparent alteration of the α1-antitrypsin's electrophoretic microheterogeneity during the course of therapy in any of the seven "responders" (example shown in Fig. 2). Thus, the five of six PiZ patients who responded to danazol did so with increases in the Z protein. Planimetric analysis of crossed immunoelectrophoretic patterns of serum before and after therapy showed that the patient with the PiM_{Duarte}Z phenotype responded with equivalent increases in the M_{Duarte} and Z proteins. Similar analysis demonstrated

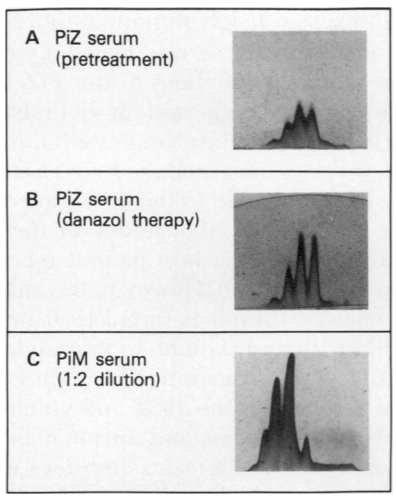

FIGURE 2 Crossed immunoisoelectrophoretic analysis of PiZ serum before (A) and 30 d after danazol therapy (B). The anode is to the left for the first dimension (isoelectric focusing). With danazol therapy the pattern remained the same but the amplitude of the peaks increased, reflecting an increase in the concentration of the PiZ protein. For comparison, a pattern for normal serum (PiM) at a 1:2 dilution is provided (C).

that in the PiSZ patient, the Z protein contributed only 30% of the total increase in serum α1-antitrypsin, whereas the S protein comprised nearly 70% (data not shown).

Quantitation of the other major serum protease inhibitors before and during danazol administration revealed no significant alteration of the group mean or individual α2-macroglobulin or antithrombin III levels in any of the homozygous or heterozygous patients ($P < 0.2$, all comparisons; Fig. 3). In comparison, serum C1 inhibitor levels increased in response to danazol; each patient demonstrated an increase in serum C1 inhibitor with group mean levels rising from 21 to 31 mg/dl, representing an average increment of 50% ($P < 0.02$). This response of serum C1 inhibitor is similar to that observed in "normal" individuals during danazol therapy (14, 15).

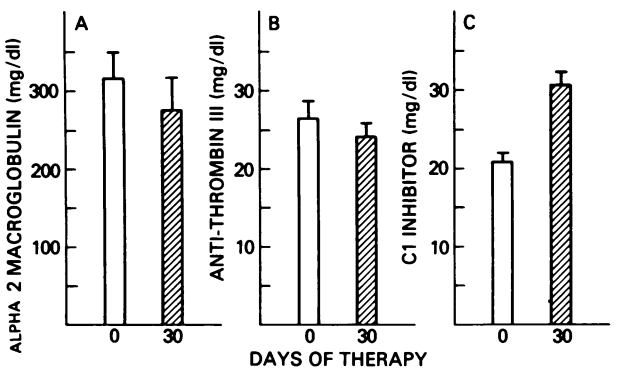

FIGURE 3 Effect of danazol administration on serum levels of antiproteases other than α1-antitrypsin in the eight study patients; (A) α2-macroglobulin, (B) antithrombin III, and (C) C1 inhibitor. Serum levels were determined before therapy (day 0) and at the completion of the 30-d danazol trial (day 30) and are expressed as the mean±SEM. There were no differences between the pre- and the posttreatment sera for α2-macroglobulin and antithrombin III ($P > 0.2$, both determinations) but C1 inhibitor levels increased significantly with danazol therapy ($P < 0.02$).

Not only did danazol increase antigenic levels of serum α1-antitrypsin in the majority of study patients, but the increased serum protein appeared to be functional as well. Assessment of serum trypsin inhibitory capacity in three of the PiZ patients (R.J., H.P., and L.E.) showed increases in α1-antitrypsin function paralleling increases in their antigenic levels (0.12±0.02 to 0.17±0.02). The one PiZ patient whose antigenic levels did not change (M.G.), also had no change in functional level. Like the PiZ patients who responded to therapy, the two heterozygous patients increased their functional serum inhibitory capacity as well as their antigenic levels.

DISCUSSION

Current concepts of the pathogenesis of the emphysema associated with serum α1-antitrypsin deficiency suggest that lung destruction in these patients results from a protease-antiprotease imbalance within the lung. In this context, such destruction potentially could be checked by therapeutic maneuvers designed to improve protease-antiprotease homeostasis within the lung parenchyma. The present study suggests that this approach may be partially realized through the administration of an impeded androgen, danazol, which produces a significant increase in serum antigenic and functional α1-antitrypsin in patients with severe deficiency of this antiprotease. In addition, the effect of danazol on α1-antitrypsin serum levels of these patients does not appear to be a general, nonspecific increased stimulation of the liver; although serum levels of C1 inhibitor were also increased, α2-macroglobulin and antithrombin III levels remained unchanged.

Significance of danazol therapy in α1-antitrypsin deficiency. Several considerations suggest that danazol may be a useful therapeutic agent in the treatment of severe α1-antitrypsin deficiency. Because the concept of lung destruction in these individuals is based on the theory of protease-antiprotease imbalance, it can be argued that any increase in circulating antiprotease may be beneficial to these individuals. Danazol increased serum levels of the PiZ patients an average of 37% and increased the serum levels of the Pi $M_{Duarte}Z$ and PiSZ individuals 85 and 87%, respectively. Although danazol did not fully restore α1-antitrypsin levels to normal, these increases may at least partially reestablish protease-antiprotease homeostasis in these patients.

Although there is no precise information regarding the threshold of serum α1-antitrypsin required for maintenance of protease-antiprotease homeostasis within the lung, estimates of this threshold level can be inferred from studies of individuals with different α1-antitrypsin phenotypes. While the PiM phenotype (serum levels 200–300 mg/dl) is not associated with increased risk of lung disease, it is estimated that 80% of PiZ individuals (levels 10–50 mg/dl) and a significant proportion of PiSZ individuals (levels 60–80 mg/dl) develop panacinar emphysema (6). In comparison, although there are anecdotal reports of PiS (levels 80–100 mg/dl) and PiMZ (levels 100–140 mg/dl) individuals with accelerated emphysema, the association of serum levels from 80–140 mg/dl with increased risk to the development of destructive lung disease remains controversial (23–31). Thus, it is likely that the threshold level of serum α1-antitrypsin necessary to maintain pulmonary protease-antiprotease homeostasis is in the range of 80 to 140 mg/dl, and that serum α1-antitrypsin levels of greater than 80 mg/dl are partially, if not completely, protective. However, additional considerations suggest that in some α1-antitrypsin deficient individuals, the serum threshold for protease-antiprotease balance may even be lower. Recent studies suggest that the protease-antiprotease equation may be influenced by the concentration of elastase in lysosomes of circulating polymorphonuclear leukocytes (32, 33), implying that individuals with relatively low levels of lysosomal elastase activity may require proportionally lower α1-antitrypsin serum levels in order to effect pulmonary protease-antiprotease homeostasis. It is conceivable, therefore, that some PiZ individuals may have an α1-antitrypsin threshold as low as 50 mg/dl, a level which is close to that reached during danazol therapy. All of these considerations must be viewed in terms of the recent epidemiologic study of smoking and nonsmoking PiZ individuals (34). Cigarette smoking is clearly a major risk in PiZ individuals and thus smoking PiZ patients are more likely to require higher α1-antitrypsin levels than nonsmokers to protect their lungs from proteolytic damage.

There has been considerable experience with the administration of danazol to normals (35), women with endometriosis (36), and individuals with hereditary angioedema (14, 15). The most common adverse effects include weight gain, water retention, acne, decreased breast size, monilial vaginitis, mild hirsutism, and menopause-like symptoms, all of which are reversible with cessation of therapy. Although it is important to integrate these adverse effects with a consideration of the risks and benefit of chronic danazol administration to α1-antitrypsin deficient patients, danazol is the first example of an agent that offers practical therapeutic potential to these individuals. Previous studies have shown that the administration of combination estrogen-progestin oral contraceptives increases serum α1-antitrypsin levels in normal individuals and those with intermediate serum deficiency of α1-antitrypsin, but produces no effect in the PiZ individual (37). Similar results are reported for diethylstilbesterol administration (13). The intravenous administration of *Salmonella typhi* vaccine produced increases in serum α1-antitrypsin comparable to those achieved by danazol in two PiZ individuals (38), as did the development of acute lobar pneumonia in a patient receiving oral contraceptive therapy (39). However, the enlistment of such inflammatory stimuli is impractical in a context wherein chronic therapy would be required. Although the oral contraceptives theoretically might raise serum α1-antitrypsin levels in the PiSZ individual, danazol is a nonvirilizing androgen, and thus may be administered to both males and females and does not appear to share a liability for thromboembolic events with the estrogen-progestin agents.

One potential hazard of chronic danazol administration relates to the probability that increases in serum α1-antitrypsin levels in these patients are the result of stimulation of the hepatocyte. Because α1-antitrypsin deficiency is associated with an inability of the hepatocyte to transport the variant protein to the extracellular space (3), if danazol stimulates hepatocytes to increase synthesis of this protein, chronic danazol administration may increase intracellular α1-antitrypsin accumulation and thus potentiate liver disease. For this reason, the present study was specifically designed to last only 30 d, a time by which early liver disease could be detected and reversed. However, no changes in liver enzymes were seen in any study patients, suggesting that this potential hazard may not be important, at least in the short term.

Future approaches. A short-term study such as this cannot answer the question as to whether danazol administration effects any alteration in lung function. However, before long-term studies to resolve this issue can be designed, three major questions must be answered. First, although danazol causes an increase in α1-antitrypsin levels within 2 wk and maintains this level for an additional 2 wk, it is not known whether these levels will be maintained during chronic therapy. Second, studies must be conducted to evaluate the effect of chronic danazol therapy on the liver function of α1-antitrypsin deficient individuals. Third, it is possible that higher levels of danazol administration may be associated with even higher levels of serum α1-antitrypsin than demonstrated in this study. In the experience with the use of danazol in the therapy of hereditary angioedema, there appears to be a dose response between the amount of drug given and the resulting serum C1-inhibitor level (15). If the same is true for α1-antitrypsin in PiZ individuals, it may be possible to raise their serum levels above 50 mg/dl with higher doses.

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