Original Paper

Therapy of CF-Patients with Amitriptyline and Placebo - a Randomised, Double-Blind, Placebo-Controlled Phase IIb Multicenter, Cohort-Study

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Key Words

Cystic Fibrosis • Amitriptyline • *P. aeruginosa* • Ceramide • CFTR

Abstract

Background/Aims: Several recent studies revealed an accumulation of ceramide in bronchial, tracheal and intestinal epithelial cells of mice and patients with cystic fibrosis (CF). Normalization of ceramide concentrations in lungs of CF mice employing the functional acid sphingomyelinase inhibitor amitriptyline also normalized mucociliary clearance, chronic inflammation and infection susceptibility to pulmonary *P. aeruginosa* in these mice. **Methods:** To test for a beneficial effect of amitriptyline *in vivo*, we performed a phase IIb randomised, double-blind, placebo-controlled study. Twenty-one CF patients were treated with 25 mg/d amitriptyline twice daily for 28 days. The placebo consisted of 19 patients and was also treated twice per day. The primary endpoint was the change in lung function in the intention-to-treat (ITT) population. Secondary endpoints were ceramide levels in epithelial cells and safety. **Results:** After treatment, forced expiratory volume in 1 sec predicted (FEV₁) increased 6.3±11.5% (p=0.08) in the ITT population (36 of 40 CF patients) and 8.5±10% (p=0.013) in the per protocol (PP) population (29 of 40 patients). Ceramide levels decreased in nasal epithelial cells after amitriptyline treatment. Amitriptyline had no severe and only mild and mostly transient adverse effects, i.e. xerostomia and tiredness. **Conclusion:** Amitriptyline is safe in CF-patients, increases FEV₁ and reduces ceramide in lung cells of CF patients.

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder in western countries and caused by mutations in the cystic fibrosis transmembranous conductance regulator (CFTR) molecule [1-3]. The disease is characterized by chronic pulmonary inflammation, increased infection susceptibility and fibrosis [4, 5]. Chronic bacterial respiratory infections have the greatest impact on morbidity and mortality of the patients [5]. Most patients suffer from chronic infections with *P. aeruginosa* [6], the major opportunistic pathogen in CF. Mucoid *P. aeruginosa* grow in the bronchi of CF-patients, are surrounded by masses of dead neutrophils [6], and high doses of antibiotics are required for treatment of CF lung disease, but even with aggressive treatment *P. aeruginosa* infections become chronic [7].

Several novel treatments including inhalation of Pulmozyme and application of CFTR correctors were introduced into the clinic within the last years [8]. However, inhalation of Pulmozyme, a DNase, does not prevent long-term decline of lung functions. CFTR correctors only promote the function of G551D mutants, while they do not promote function of CFTR in the vast majority of the patients [8].

Therefore, novel and improved treatments for CF are urgently required. Recent animal studies offer an attractive novel possibility to treat cystic fibrosis [9]: We demonstrated in mice genetically deficient for *Cftr* an accumulation of ceramide in tracheal and bronchial epithelial cells [9]. The results were recently independently confirmed and transferred to the human situation demonstrating accumulation of ceramide in bronchial epithelial cells of patients with cystic fibrosis [9-12]. Ceramide mediates increased death of bronchial epithelial cells resulting in a release of dead cells and finally DNA into the bronchial lumen [9]. The reduction of the mucociliary clearance by ceramide facilitates infections with *P. aeruginosa* [9]. In addition, ceramide induces chronic inflammation in CF lungs [9]. Genetic or pharmacological inhibition of acid sphingomyelinase (ASM), which releases ceramide, reduced pulmonary ceramide levels and normalized mucociliary clearance, inflammation and infection susceptibility [9]. Inhibition of the acid sphingomyelinase was achieved by treatment of CF mice with amitriptyline, a known functional blocker of the acid sphingomyelinase [13, 14].

These very encouraging animal data prompted us to test for a beneficial effect of amitriptyline in CF patients. Amitriptyline is well established in treatment of patients with major depression and clinical studies are warranted to assess tolerability and efficacy of amitriptyline for treatment of CF patients. We have previously assessed the therapeutic efficacy and safety of amitriptyline in a pilot study involving 4 CF patients treated with amitriptyline or placebo demonstrating a significant increase in lung function after 2 weeks of treatment with amitriptyline. We subsequently performed a phase IIa study for safety, proof-of-mechanism and dose-finding study involving 19 CF patients [15]. This study revealed that amitriptyline is safe in CF patients, since only mild adverse effects were observed. Moreover, the per protocol (PP) analysis of this study demonstrated a 5% increase of the forced expiratory volume in one second (FEV₁) after treatment with amitriptyline compared to the placebo group suggesting an improvement of lung functions by amitriptyline [15]. Although it is difficult to exactly determine the complex physiology and pathophysiology of the lung in CF patients, medical associations (European Medicines Agency, Food and Drug Administration, USA) suggested to use the FEV₁, i.e. the maximal volume exhaled by patient within the 1st second after maximal inhalation, as the most appropriate parameter to determine lung function.

Here we performed a phase IIb study on 40 CF-patients treated with 25 mg amitriptyline twice daily. We demonstrate an increase of FEV₁ in the intention-to-treat (ITT) and PP populations upon treatment of CF patients with amitriptyline for four weeks. The side effects of the treatment are minor and amitriptyline is well tolerated by CF patients.

Methods

Study design

We performed a phase IIb study in a multicentric, randomised, double-blind, placebo-controlled, cohort study design in 40 CF patients, attending the CF centre of the Justus-Liebig-University Giessen, the CF centre of the Children's University Hospital of Jena and the CF centre of the Children's University Hospital of Tübingen, Germany. The study was approved by the Institutional Review Board of the University Hospital Tübingen. All patients and parents of adolescent patients gave written informed consent and were insured for potential adverse effects. The phase IIb study was registered at EudraCT 2008-002673-13 and ClinicalTrials.gov, number NCT01309178.

Patients

Patients were included in the study based on the following criteria: cystic fibrosis was verified; patient was older than 14 years; patient's weight was more than 35 kg; FEV₁ was higher than 30% and lower than 90%; patient was pulmonary colonized with bacteria; no acute pulmonary illness was present; C-reactive protein (CRP) was not elevated two fold (2 mg/dl) over normal levels; lung function tests were possible and a full course of therapy was possible without any restrictions. Patients were excluded from the study based on the following criteria: FEV₁ in baseline differed more than 10% from screening visit; CRP in baseline differed more than 50% from screening visit; glaucoma, seizures, heart insufficiency or major depression were present; intravenous antibiotic treatment was necessary in the last 4 weeks before inclusion visit; high dose steroid therapy was necessary; on/off-therapy of tobramycin was present in the last 2 weeks; involvement of the patient in another study; pregnancy and nursing mothers. Patients were randomized to amitriptyline capsules, administered orally at a dosage of 25 mg amitriptyline per capsule, twice daily for 4 weeks, or the placebo (corn starch capsules) using the software StatsDirect 2.7.2 (StatsDirect Ltd; Altrincham, Cheshire, UK). During treatment period, patients' visits were at days -7, 0, 14 and 28. A follow up visit was initiated at day 36.

Outcome

The primary outcome was the difference of FEV₁ predicted at day 28 to placebo in the ITT population measured by spirometry (Jaeger, Höchberg, Germany). Secondary endpoints were (i) absolute and relative FEV₁ differences to baseline, (ii) ceramide concentrations in respiratory epithelial cells, (iii) bacterial counts, (iv) leukocyte counts, (v) concentrations of cytokines and (vi) DNA concentrations in sputum specimens. C-reactive protein (CRP) and the cytokines IL-1 β , IL-6, IL-8, IL-10 were determined in blood and sputum specimens using routine methods and the Bio-Plex 200 System (Bio-Rad Laboratories GmbH, München, Germany). C-reactive protein was measured as an additional inflammatory parameter in blood specimens using luminescence technology. Nasal epithelial cells were obtained from all patients for the semi-quantitative determination of ceramide using immunofluorescence [9]. To this end, the cells were stained with a Cy3-coupled monoclonal anti-ceramide antibody (Glycobiotech) and analysed by fluorescence microscopy. The relative amount of ceramide was determined by measuring the fluorescence intensity on a Leica fluorescence microscope DMIRE 2. The intensity of the staining was scored from weak to strong staining. DNA and number of bacterial pathogens were quantified electrophoretically [16] and by routine culturing on blood agar, respectively, at day 28.

For assessing safety of amitriptyline, blood pressure, heart rate, transaminases and creatinine values were measured at each visit. An electrocardiogram was taken at day 14. Adverse effects (AEs) and severe AEs (SAEs) were documented on patient sheets.

Statistics

Based on the results of our previous studies [15], we used the difference of relative FEV₁ predicted between cases and controls after treatment of 28 days as primary endpoint.

Due to the fact, that FEV₁ is measured as %, geometric means were used to compare the results in the verum and the placebo group by student's t-test. Confirmative analysis was based on the ITT-population, with significance level $\alpha=0.05$. Secondary endpoints were FEV₁ predicted absolute in the ITT and absolute and relative to baseline in the PP population and ceramide concentration. The latter is presented by frequencies and 95%-confidence intervals of patients with decrease in ceramide concentration after

Fig. 1. CONSORT patient flow chart. Screening and randomization. *All CF patients older than 14 years admitted to the CF centres were assessed for eligibility. ITT: Intention to treat; PP: Per protocol. CRP: C-reactive protein.

treatment. Analysis of secondary endpoints or analysis based on the PP population was regarded as being descriptive.

The ITT analysis includes all patients who participated in the study, regardless any protocol violations. Even a patient, who was accidentally treated with the placebo instead with amitriptyline as happened in the present study, is included in the ITT analysis.

The PP population excluded all patients that did not take study medication according to the study protocol or showed high changes in CRP. Missing values were not replaced.

Results

Ninety-five patients were initially screened for the study. From the 40 adolescent and adult CF patients for this phase IIb study, 21 patients were randomized to receive amitriptyline and 19 patients to receive placebo treatment. Two patients were excluded from analysis due to high CRP and one patient due to missing lung function values at day 28. The remaining 37 patients were included in the ITT analysis (19 females, 18 males; mean age: 25.5 ± 8.4 yrs) (Fig. 1). A further patient refused to take medication. Due to a mistake in randomisation, one patient in the amitriptyline group received placebo and one patient in the placebo group received amitriptyline. For PP analysis, seven patients were excluded during the study due to high CRP or not correctly medicated. Twentynine patients could be included in the per-protocol analysis (PP), 16 patients who received amitriptyline and 13 received placebo were analysed after correction of the randomisation mistake (Fig. 1). The demographic data of the patients (Table 1) reveal that both groups were similar with regard to gender, age, weight, lung function parameters, pancreatic sufficiency, and chronic bacterial infections.

After 28 days of amitriptyline treatment, lung functions of CF patients, measured as FEV₁, predicted absolute and relative to baseline, improved in the ITT population to 0.6% and 0.2%, respectively (Table 2). In contrast, placebo treatment resulted in a decrease of FEV₁ in the ITT population of 3.8% and 6.1%, respectively. The difference of FEV₁ predicted absolute

	Amitriptyline	Placebo	
Characteristic	(n = 19)	(n = 18)	p value
Female Gender (%)	9 (42)	10 (56)	0.44
Age (years) a	25.1±8.2	25.8±8.9	0.32
Weight (kg) *	56.9±8.9	53.0±6.5	0.23
FEV ₁ % *	65.0±15.6	58.4±17.7	0.24
FVC % *	81.4±13.3	75.5±12.8	0.19
MEF ₂₅₋₇₅ % *	35.4±17.7	30.6±23.4	0.51
Pancreas insufficiency (%)	2 (10.5)	0	n.a.
<i>P. aeruginosa</i> , non mucoid (mean logcfu)	8 (7.5)	8 (6.7)	0.44
<i>P. aeruginosa</i> , mucoid (mean logcfu)	7 (6.4)	7 (6.9)	0.5
<i>St. aureus</i> (mean logcfu)	10 (6.3)	10 (6.6)	0.5
MRSA	1	0	n.a.
<i>Stenotrophomonas maltophilia</i> (mean logcfu)	6 (5.8)	2 (7.7)	0.06
<i>Aspergillus fumigatus</i>	3	3	0.5
<i>Candida albicans</i>	7	3	0.09
<i>E. coli</i>	1	1	n.a.
others	3	3	n.a.

a Values are means ± SD; MRSA: methicillin resistant *Staphylococcus aureus*

Table 1. Demographic data and baseline characteristics in cystic fibrosis patients treated in the phase IIb amitriptyline study. Presented are the characteristics of all included individuals, i.e. gender, age, weight, the forced expiratory volume in the 1st second (FEV₁), the forced vital capacity (FVC), the expiratory flow at 25, 50 and 75% of the forced vital capacity (MEF₂₅₋₇₅), the percentage of patients with pancreas insufficiency, the logarithmic average of colony forming units (logcfu) of *P. aeruginosa*, *S. aureus*, multiple resistant *S. aureus*, *S. maltophilia*, *A. fumigatus*, *Candida albicans*, *E. coli* and other, not further specified bacteria in the lung.

Population	Treatment group	Number of patients	FEV ₁ (% predicted)			
			Absolute to baseline	p value	Relative to baseline	p value
ITT	Amitriptyline	19	+0.6±5.7*	0.034	+0.2±11.4	0.08
	Placebo	17	-3.8±6.9		-6.1±11.5	
PP	Amitriptyline	16	+2.2±5.2	0.013	+3.6±7.9	0.013
	Placebo	13	-2.7±5.0		-4.9±10.1	

* values represent geometric means ± standard deviations. Student's t-test was used

Table 2. Efficacy of amitriptyline in patients with cystic fibrosis. Lung function was determined in the Intention to treat (ITT) and the per protocol (PP) population as forced expiratory volume in one second (FEV₁) after 28 days of amitriptyline or placebo treatment in a phase IIb cohort study. The number (n) of patients in the different groups is given. Values represent geometric means ± standard deviations. Significant difference of FEV₁ between placebo and amitriptyline-treated groups are indicated by an asterisk (*), p≤0.05, Student's t-test.

to baseline between amitriptyline and placebo was statistically significant (p=0.03), while the primary endpoint, i.e., FEV₁ predicted relative to baseline, was not significant (p=0.08) (Table 2). Five of 19 patients in the placebo group showed a 10% or more decline of FEV₁ after 28 days of therapy because of lower respiratory tract infections, whereas no patient with such a high decline was found in the amitriptyline group.

The PP analysis revealed a significant increase of the primary endpoint, i.e. both FEV₁ predicted absolute and relative to baseline improved significantly by 4.9% (p=0.013) and 8.5% (p=0.013), respectively (Table 2).

The PP analysis also demonstrated a decrease of the secondary endpoint ceramide-levels in airway epithelial cells after 28 days of treatment with amitriptyline. The relative fluorescence intensities for ceramide in epithelial cells were scored by a blinded investigator with weak (1), mediate (2), strong (3) and very strong (4). The relative fluorescence intensity

	Amitriptyline	Placebo	
Adverse effects *	(n = 45)	(n = 23)	p value**
Xerostomia (%)	14 (82)	3 (18)	0.053
transient	9	3	
Tiredness (%)	17 (71)	7 (29)	0.28
transient	12	6	
Exacerbation	0	1	0.08
Common cold (%)	11 (65)	6 (35)	0.44
Headache (%)	1 (33)	2 (67)	0.11
Gastroenteritis (%)	1 (33)	2 (67)	0.11
Pruritus	0	1	0.08
Dizziness	1	0	0.24
Sleep problems	0	1	0.08

Table 3. Safety of amitriptyline in CF patients during the phase IIb amitriptyline study*. Presented are the adverse effects observed in the present study in the amitriptyline and the placebo groups. Sixty-eight adverse effects were observed in 30 out of 40 patients. *: The safety of amitriptyline was assessed after each treatment course of 28 day; **: Student's t-test was used for all statistical determinations, $p \leq 0.05$.

for ceramide in the PP population decreased in 9 of 14 patients (64%; 95%-confidence interval (CI) 35-87%) in the amitriptyline group. However, we also observed a change of ceramide intensities in 6 of 13 patients (46%; 95%-CI 19-75%) of the placebo group after 28 days of treatment. The other secondary endpoints, i.e., bacterial counts, leukocyte counts, concentrations of cytokines and DNA concentrations in sputum specimens of CF patients were not significantly different between the amitriptyline and the placebo group (data not shown).

Amitriptyline was well-received in the patient group and no SAE was observed in the amitriptyline-treated group. One SAE was recorded at the end of the 28 day course in the placebo group. Sixty-eight AEs were observed in 30 out of 40 patients. Thirty-one AEs were related or possibly related to the medication. The incidence of two well-known AEs of amitriptyline, xerostomia and tiredness [14, 15], were different between the placebo and the amitriptyline group. However, the effect was mostly transient (Table 3). None of the other typical adverse effects of amitriptyline, i.e., vertigo, arterial hypotonia, long QT syndrome, tremor, dyskinesia, obstipation, dysuria or glaucoma were observed in our study, probably because of the low dose administered. Taken together, our data demonstrate a positive trend of amitriptyline to increase lung function and decrease ceramide levels in CF patients.

Discussion

In the present study, we tested whether amitriptyline improves lung functions in patients with CF. Since data from our phase IIa study [15] indicated that amitriptyline therapy using 25 to 75 mg given twice daily for 14 days is safe and increases FEV_1 , we performed the present phase IIb trial to evaluate the effect of amitriptyline on FEV_1 in a ITT and PP protocol. Further, the present study served to define the number of patients required for a phase III study using ITT population.

The present phase IIb study demonstrates a significant increase of the lung function of CF-patients after treatment with amitriptyline in the PP population. The positive effect of amitriptyline on lung function is supported by the finding that ceramide levels determined in airway epithelial cells decreased under amitriptyline therapy. However, future studies require a more quantitative analysis of ceramide, for instance analysis of the samples by mass spectrometry or ceramide kinase assays. Further, it might be better to use nasal brushes to obtain clearly-defined cell populations instead of sputum.

The study also demonstrates that amitriptyline, a well-established agent in treatment of major depression in adults [14], is safe at a dose of 2 x 25 mg/day in all CF patients when

given orally for a period up to four weeks. No severe adverse drug reactions were reported, while typical AEs related to amitriptyline such as xerostomia and tiredness occurred. Importantly, AEs were mostly transitory and occurred only during the first days of therapy. Although CF patients seem to have an altered metabolism in general [17], the frequency and nature of side effects related to amitriptyline in those patients did not differ from reported adverse drug reactions of the drug in non-CF patients.

A further concern for the use of amitriptyline in CF patients infected chronically with *P. aeruginosa* or other bacterial pathogens, is the fact that amitriptyline might prevent the acute increase of ceramide levels in the respiratory tract observed after *P. aeruginosa* infections, which seems to be part of the host defence [18]. However, amitriptyline-treatment normalises, but does not reduce ceramide levels in cystic fibrosis lungs leaving the local response to *P. aeruginosa* intact [9, 18]. Accordingly, the number of acute exacerbations did not increase significantly in the amitriptyline group compared to controls demonstrating again the safety of the applied amitriptyline dose in the CF patient study group. The observation that 5 of 19 patients in the placebo group showed a more than 10%-decline of FEV₁ after 28 days observation contrasts to a decline of 1 - 2 % per year reported by other studies [5] and might be caused by the relatively short observation period in the present study. However, such a high decline was not seen in the amitriptyline-treatment group in the present study treated in parallel and this finding may support the notion of a beneficial effect of amitriptyline on lung functions.

Ceramide might have several actions in the lung. First, increased ceramide concentrations in bronchial epithelial cells were shown to trigger cell death and to mediate a release of DNA into the airways [9]. DNA reduces the mucociliary clearance and facilitates infections with *P. aeruginosa* [9]. Further, ceramide was shown to increase the pulmonary concentrations of pro-inflammatory mediators and trigger inflammation. The molecular mechanisms of this effect are presently unknown. All of these changes are prevented by normalization of pulmonary ceramide concentrations. Thus, the effects of amitriptyline on FEV₁ observed in the present study might be caused by a combination of normalization of epithelial cell death and mucociliary clearance and a reduction of pulmonary inflammation.

In summary, the present findings demonstrate the safety and the efficacy of a treatment of CF patients with a low dose of amitriptyline and justify the continuation of the development of amitriptyline as a new strategy to treat CF patients in a future phase III study. It should be pointed out that an increase of 5-8% of lung function (FEV₁) is comparable to the beneficial effects of other drugs commonly used to treat cystic fibrosis. Furthermore, the potential of drugs that target the acid sphingomyelinase in cystic fibrosis might be increased by more efficient inhibition of the acid sphingomyelinase and/or local pulmonary application of higher doses without systemic effects.

Conflict of interest

We declare that we have no conflict of interest.

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JR and EG planned the project. LN, JGM and JR conducted the study. JR conceived the project, was principal investigator, and co-wrote the paper with EG. CE contributed to the project design and analysed the data. GH, VI, CD, AW and KU analysed data and tested all

patient samples in the various assays. EG, JL and CA tested ceramide levels. UGM, AH, SH and MS screened patients. GD reviewed the manuscript.

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