Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2009;24:65-72

Accepted: May 11, 2009

Therapeutic Efficacy and Safety of Amitriptyline in Patients with Cystic Fibrosis

Joachim Riethmüller[1], Janina Anthonysamy[1], Emilio Serra[1], Matthias Schwab[2], Gerd Döring[3,*] and Erich Gulbins[4,*]

[1]Department of Paediatrics, University Hospital Tuebingen, Tuebingen, [2]Dr Margarete Fischer Bosch Institute of Clinical Pharmacology, Stuttgart, and Department of Clinical Pharmacology, University Hospital Tuebingen, Tuebingen, [3]Department of Clinical Microbiology and Hygiene, University Hospital Tuebingen, Tuebingen, [4]Department of Molecular Biology, University of Duisburg-Essen, Essen, *these authors contributed equally to the manuscript and share senior authorship

Key Words

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

Abstract

Amitriptyline, a blocker of acid sphingomyelinase and acid ceramidase, significantly reduces *Pseudomonas aeruginosa* lung infection in cystic fibrosis (CF) mice with concurrent increase of survival. Our aim was to establish whether amitriptyline is safe and effective in the treatment of CF patients. In a randomised, double-blinded, placebo-controlled, cross-over pilot study, 4 adult CF patients received 37.5 mg of amitriptyline or placebo twice daily for 14 days. Subsequently in a phase II study 19 adult CF patients were randomly allocated to three treatment groups receiving amitriptyline once daily for 28 days at doses of 25 mg (n=7), 50 mg (n=8), or 75 mg (n=8) or placebo (n=13). The primary outcome was the difference of forced expiratory volume in 1 sec (FEV_1) at day 14 between amitriptyline and placebo. Primary endpoint measures improved significantly in three of four patients in the pilot study after amitriptyline treatment vs placebo (relative FEV_1: 14.7±5%; p = 0.006) and in the 25 mg treatment group of the phase II study (relative FEV_1: 4.0±7%; p = 0.048). Amitriptyline was well tolerated in both studies and 96% of the patients completed the studies. Amitriptyline as a novel therapeutic option in patients with CF is safe and seems to be efficacious.

Introduction

The hereditary disease cystic fibrosis (CF), caused by mutations in the gene encoding the CF Transmembrane conductance Regulator (*CFTR*) [1-3], affect epithelial ion and water transport in cells in the respiratory, gastrointestinal, hepatobiliary and reproductive tracts. Reduced chloride secretion causes a viscous mucus, overlaying the respiratory epithelium, leading to impaired mucociliary clearance [4]. This supports chronic bacterial respiratory infections, which have the greatest impact on morbidity and mortality of the patients [5]. Mostly due to the endobronchial location of mucoid *Pseudomonas aeruginosa* [6], the major opportunistic pathogen in CF,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/09/0242-0065$26.00/0

Accessible online at:
www.karger.com/cpb

Dr. med. Joachim Riethmueller
Department of Paediatrics, University Hospital Tuebingen
Hoppe-Seyler Str. 1, D- 72076 Tübingen, (Germany)
Tel. + 49 7071 2981391, Fax +49 7071 294450
E-Mail joachim.riethmueller@med.uni-tuebingen.de

which is surrounded by masses of dead neutrophils [6], high doses of antibiotics are required for treatment of CF lung disease [7]. Nevertheless, once *P. aeruginosa* infections become chronic, eradication by antibiotics cannot be any more accomplished [7]. Thus, other strategies have to be established.

Recent animal studies offer an attractive alternative. We demonstrated in mice, genetically deficient for *Cftr*, that cells of the respiratory tract accumulate ceramide in an age-dependent manner [8]. Accumulation of ceramide was caused by an imbalance between the activities of the acid sphingomyelinase that produces ceramide by sphingomyelin hydrolysis, and the acid ceramidase that degrades ceramide to sphingosine. Ceramide accumulation resulted in increased inflammation in the lung, which was normalised by inhibition of the acid sphingomyelinase and correction of pulmonary ceramide concentrations. Further, we observed increased death of respiratory epithelial cells in *cftr* deficient mice that resulted in deposition of DNA on the respiratory epithelium. These DNA deposits facilitated adhesion of and infection of the mice with *P. aeruginosa* [8]. Treatment of the CF mice with amitriptyline, a blocker of acid sphingomyelinase [9, 10] and acid ceramidase, prevented cell death and protected the animals from severe pulmonary *P. aeruginosa* infections. Furthermore, amitriptyline almost completely protected these mice from lethal *P. aeruginosa* infections [8].

Although these animal data are encouraging and amitriptyline is well established in treatment of patients with major depression, clinical studies are warranted to assess tolerability and efficacy of amitriptyline for this novel indication. For instance, studies have revealed disease-specific increases in the formation of drug metabolites [11], and therefore higher concentrations of toxic metabolites cannot be excluded. Furthermore, the use of amitriptyline in CF patients, infected chronically with *P. aeruginosa* or other bacterial pathogens, may negatively affect host defence [12]. Therefore, we assessed the therapeutic efficacy and safety of amitriptyline in patients with CF using different dosages of amitriptyline in a pilot and subsequently in a phase IIa study.

Materials and Methods

Study design and patients

We carried out a pilot and phase IIa study in a monocentric, randomised, double-blinded, placebo-controlled, cross-over study design in 23 CF patients, attending the CF centre of the Children´s University Hospital of Tuebingen, Tuebingen, Germany. Both studies were approved by our Institutional Review Board of the University Hospital. All patients gave written informed consent and were insured for potential adverse effects. The phase IIa study was registered at EudraCT 2006-002259-33 and ClinicalTrials.gov, number NCT00515229.

Patient No	Microbiology	FEV_1 (%)
1	BC, Serratia	48
2	Ps, MRSA	74
3	BC	33
4	Sphingomonas	102
5	Ps.	80
6	Ps.	20
7	Ps., Stenotrophomonas	71
8	Ps., MRSA	51
9	Ps.	28
10	Ps., Sphingomonas	76
11	Ps.	37
12	Citrobacter, Klebsiella	86
13	Stenotrophomonas	56
14	Sphingomonas	82
15	Ps., Sphingomonas	39
16	Ps.	52
17	Proteus vulgaris	72
18	Ps, MRSA	50
19	Ps, MRSA	90

Table 1. Baseline data for microbiology and FEV_1 of 19 cystic fibrosis patients in a phase IIa cross-over study. BC: *Burkholderia cepacia;* MRSA: methicillin resistant *Staph. aureus;* Ps.: *Pseudomonas aeruginosa.*

Pilot study

Four adult CF patients (1 female, 3 males; mean age 28.5 ± 10 yrs; FEV_1: median: 60.5% predictive) were included in the study based on the following criteria: age >18 yrs and the presence of chronic pulmonary infection with *P. aeruginosa*, *Staphylococcus aureus* and other bacterial pathogens. Because mutations of cytochrome P450 (CYP2D6) may reduce the hepatic metabolism of amitriptyline, patients were excluded if they were homozygous or compound heterozygous for the *CYP2D6* alleles **4,*5,*6,*9,*10,*41*, since these individuals may have a higher sensitivity to amitriptyline. Genotyping was performed as previously described [13]. Glaucoma, seizures, heart failure, major depression, clinical instability, pregnancy or involvement in other clinical studies were further exclusion criteria. Clinical instability was diagnosed when symptoms of bronchitis or lar-

Table 2. Treatment courses of three amitriptyline doses and placebo in 19 cystic fibrosis patients in a phase IIa cross-over study. *: Corn starch; **: Patients received placebo, 25 mg/d and 50 mg/d amitriptyline, or placebo, 25 mg/d and 75 mg/d amitriptyline, or placebo, 50 mg/d and 75 mg/d amitriptyline. In case primary endpoint data from treatment courses were missing, patients were re-treated with the same dosage on blinded conditions at the individual end of the study (additional courses); D: dose reduction or interruption; E: exacerbation.

Patient No.	Treatment courses							
	Placebo*	Amitriptyline [mg]						
		Courses**			Additional courses**			
		25	50	75	25	50	75	
1	+	+	+					
2	+		+	D				
3	+	D, E		+				
4	+	+		+				
5	+	+		+				
6	+	D	+					
7	+	+	+					
8	+	D		D	D			
9	+		D	D		D, E		
10			D					
11	+	+	+					
12	+	+		+				
13	+		+	+			+	
14	+		D	D		D		
15	+		+	+				
16	+	+	+					
17	+	E		E			+	
18	+		+	E			+	
19	E	+						

yngitis were noticed during the study. All patients received 37.5 mg amitriptyline or placebo twice daily for two weeks, followed by a wash-out phase of two weeks. Patients were randomized using the software JMP (JMP 5.0.1.2, SAS Institut Inc., Cary NC, USA , 2003). During both treatment periods, patients' visits were at days 0, 7 and 14. A follow up visit was initiated at day 56. The primary outcome was the relative difference of forced expiratory volume in 1 sec (FEV_1) at day 14 relative to placebo, measured by spirometry (Jaeger, Höchberg, Germany).

For assessing safety of amitriptyline, blood pressure, heart rate, transaminases and creatinine values were measured at each visit. An electrocardiogram was taken at day 7. Adverse effects (AEs) and severe AEs (SAEs) were documented on patient sheets.

Phase IIa Study

Twenty-one CF patients were screened using the above mentioned inclusion and exclusion criteria. Nineteen patients were finally enrolled (10 females, 9 males; mean age: 28.3 ± 10 yrs; FEV_1: median: 61% predictive), five of whom suffered from CF-related diabetes and one from psoriasis. All patients were chronically infected by various bacterial pathogens including *P. aeruginosa*, *S. aureus, Burkholderia cepacia* complex strains, Sphingomonas, Citrobacter, Klebsiella spp. or *Stenotrophomonas maltophilia*. The lung function of the patients was highly heterogenous at baseline and FEV_1 differed from 20% to 110% (Table 1). For ethical reasons we had to investigate adult patients and were unable to perform studies on small children that may not be pulmonary colonized with bacterial pathogens. Patients were excluded if they were homozygous or compound heterozygous for the *CYP2D6* alleles **4,*5,*6,*9,*10,*41*.

Using JMP (JMP 5.0.1.2, SAS Institut Inc., Cary NC, USA, 2003), the patients were randomly allocated to three treatment groups receiving 2 doses of amitriptyline or placebo once daily for 28 days. Six patients received placebo, 25 mg and 50 mg amitriptyline, six patients received placebo, 25 mg and 75 mg amitriptyline, and another six patients received placebo, 50 mg and 75 mg of amitriptyline (Table 2). During the three treatment courses, patients' visits were at days 0, 7, 14 and 28. When no data of visit at day 14 were available, patients were re-treated with the same dosage on blinded conditions at the individual study end. The latter was performed with one patient receiving 25 mg, two patients receiving 50 mg and in three patients receiving 75 mg of amitriptyline. A follow up visit was performed at day 165 (Table 2).

The primary outcome was the difference of FEV_1 relative to placebo at day 14 in the per-protocol (PP) group measured by spirometry (Jaeger) analogue to the pilot study. We collected blood, sputum and nasal epithelial cells for testing several secondary endpoints at day 14 of placebo or amitriptyline treatment: (i) absolute and relative FEV_1 differences, (ii) ceramide concentrations in respiratory epithelial cells, (iii) leukocyte counts in sputum specimens, (iv) bacterial counts in sputum, and (v) concentrations of the cytokines in sputum and plasma and (vi) DNA concentrations in sputum specimens. Additionally, at day 14 leukocytes, C-reactive protein and the cytokines IL-1β, IL-6, IL-8, IL-10 were determined in blood and sputum specimens using routine methods and the Bio-Plex 200 System

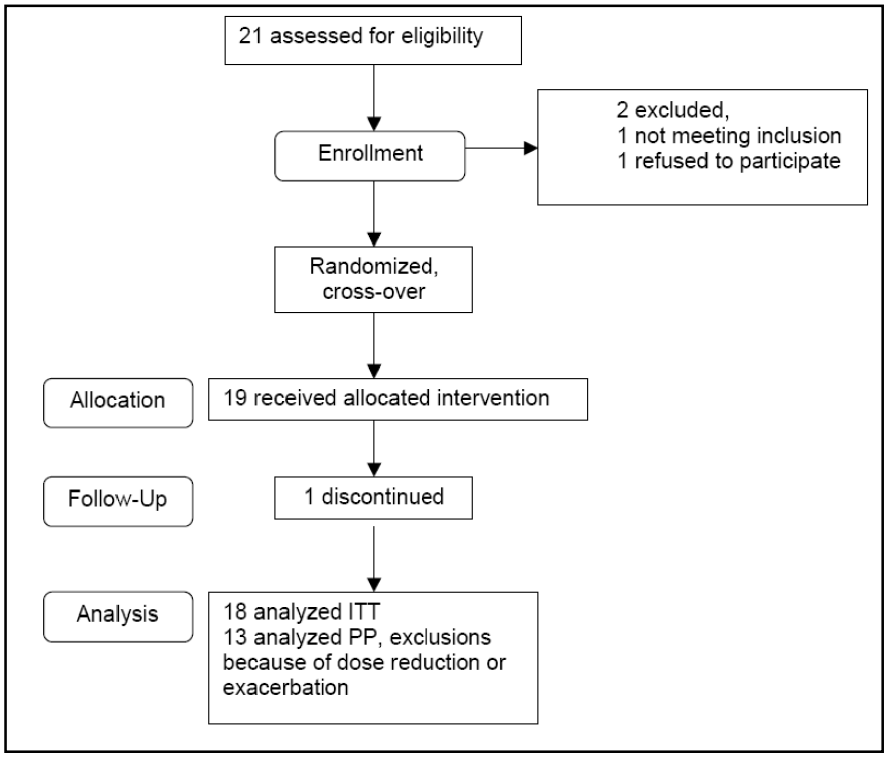

Fig. 1. Flowchart of the phase IIa amitriptyline study in cystic fibrosis patients. Twenty-one CF patients were screened, two patients refused, one patient discontinued treatment and 18 patients completed the study.

(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 [8]. DNA and bacterial pathogens numbers were quantified electrophoretically [14] and by routine culturing on blood agar, respectively, at day 14.

Ceramide was determined by staining epithelial cells in the sputum of the patients with Cy3-coupled anti-ceramide antibodies. The relative amount of ceramide was determined by measuring the fluorescence intensity on a Leica fluorescence microscope DMIRE 2.

The safety of amitriptyline was assessed in all patients after 28 days of treatment.

Statistics

Based on the results of our pilot study, the difference of FEV_1 between cases and controls after treatment of 14 days was used as primary endpoint. The primary endpoint was analysed by per protocol (PP) analysis to permit the exclusion of patients suffering from infections according to the exclusion criteria. The safety of amitriptyline was determined by intention to treat (ITT) analysis. If only a single FEV_1 value was available, data could not be used for analysis. Also, if data from two of the three courses of a patient were missing, data of this patient were not considered for ITT and PP analysis. Furthermore, patient data from therapy courses in which the dose of amitriptyline was decreased to 50% due to AEs, or in which treatment was discontinued due to AEs were not included in the PP analysis. Lastly, data from patients who became clinically instable and had to be treated by oral or intravenous antibiotics were excluded from the PP analysis. For safety all courses were considered for ITT analysis. A multivariate u-score [15] was used to evaluate secondary endpoints. Patient data were analyzed after closure of the data base using the Magic software (Univ. Tübingen, Germany), JMP (JMP 5.0.1.2, SAS Institut Inc., Cary, NC USA, 2003) and mustat (Software, version 3.0, interactively used at www.mustat.rockefeller.edu). The data analysis included a calculation of a carry over-effect using [16].

Monitoring

The study was monitored by CenTrial gGmbH, Tübingen, Germany.

Results

We have previously shown that ceramide is increased in the lungs of cystic fibrosis mice [8]. Treatment of the mice with amitriptyline normalized pulmonary ceramide concentrations and, most important, reduced lung inflammation and infection susceptibility of these mice. Here, we performed two smaller clinical studies to apply these insights for the treatment of cystic fibrosis patients.

In the first study, we investigated 4 adult patients who received either 37.5 mg amitriptyline or placebo twice daily in a cross over design interrupted by a two week phase.

Table 3. Efficacy of amitriptyline in patients with cystic fibrosis. Lung function was determined in the Intention to treat (ITT) and the per protocol (PP) group as forced expiratory volume in one second (FEV_1) after 14 days of amitriptyline or placebo treatment in a phase IIa cross-over study. *: Student's t-test was used for all statistical determinations; **: slopes were determined according to ref. 20; ***: values represent means ± standard deviations.

Treatment group		No of patients	Dose of Amitriptyline [mg]	FEV_1			P value*
				Absolute	Relative [% predicted]***	Slopes**	
ITT	Placebo	18	0	-1.2±6	-0.9±11	-0.4±3	
	Amitriptyline						
		13	25	-1.9±8	-3.8±13	-1.0±4	0.76
		13	50	-0.9±4	-2.0±9	-0.4±2	0.87
		14	75	-1.8±4	-2.5±8	-0.6±2	0.75
PP	Placebo	13	0	-1.2±4	-1.0±8	-0.6±2	
	Amitriptyline						
		7	25	+3.0±4	+4.0±7	+1.5±2	0.048
		8	50	+0.7±3	+0.6±9	+0.4±2	0.28
		8	75	-0.7±4	-0.06±9	+0.3±2	0.79
		23	25-75	+1.0±4	+1.5±8	+0.7±2	0.07

Table 4. Safety of amitriptyline in CF patients during the phase IIa amitriptyline study*. *: safety of amitriptyline was assessed after each treatment course of 28 day; **: Student's t-test was used for all statistical determinations; ***: placebo vs 25 mg/d, 50 mg/d and 75 mg/d of amitriptyline; ****: non significant for all drug concentrations against placebo.

Adverse effects	Placebo	Amitriptyline			P value**
		25 mg/d	50 mg/d	75 mg/d	
	Number of adverse effects (% of patients)				
Xerostomia	1 (6)	6 (46)	11 (79)	9 (60)	<0.01***
transient	1 (100)	5 (83)	7 (64)	6 (67)	
Tiredness	2 (11)	4 (31)	11 (79)	6 (40)	<0.05***
transient	2 (100)	3 (75)	4 (36)	3 (50)	
Vertigo	1 (6)	0 (0)	1 (7)	1 (7)	n.s.****
Exacerbations	2 (11)	2 (15)	1 (7)	2 (13)	n.s.
Pulmonary obstruction	0 (0)	0 (0)	0 (0)	1 (7)	n.s.
Back pain	1 (6)	0 (0)	0 (0)	1 (7)	n.s.
Headache	0 (0)	1 (8)	1 (7)	0 (0)	n.s.
Dysuria	0 (0)	0 (0)	0 (0)	1 (7)	n.s.
Obstipation	0 (0)	0 (0)	1 (7)	1 (7)	n.s.
Exanthema	0 (0)	0 (0)	0 (0)	1 (7)	n.s.
Stomach pain	1 (6)	0 (0)	0 (0)	0 (0)	n.s.
Gastroenteritis	0 (0)	1 (8)	0 (0)	0 (0)	n.s.

After 14 days of treatment, the primary endpoint FEV_1 had improved significantly in three of the four patients of the pilot study relative to placebo (FEV_1: 16.7%; $p = 0.006$). Data from one patient in the treatment group and one patient in the placebo group were not included due to drug-unrelated adverse effects, i.e. lower respiratory tract infections. Amitriptyline was well received in the patient group and no SAEs were recorded at the end of the 14 day courses. The preliminary data of this study prompted us to further evaluate amitriptyline in a larger cohort of CF patients.

From the 21 adult CF patients, screened for this phase IIa study, one CF patient, positive for a mutation in the *CYP2D6* gene, was excluded [17]. Another screened patient refused to participate in the study (Figure 1). The remaining 19 patients enrolled for the study, were randomized to receive placebo and two of the three amitriptyline doses in a randomized cross-over design for 84 days (Table 2), i.e. patients received either placebo for 28 days and 25 mg of amitriptyline for 28 days and 50 mg for 28 days, or placebo for 28 days and 25 mg of amitriptyline for 28 days and 75 mg for 28 days, or placebo for 28 days and 50 mg of amitriptyline for 28 days and 75 mg for 28 days. One patient discontinued treatment after 10 days due to an AE and 18 patients finished the study. All patients suffered from chronic bacterial infections (Table 1). The FEV_1 values ranged from 20% to 100% (Table 1).

During the phase IIa study, 6 of the 19 patients were excluded from analysis according to the criteria of the study plan. Therefore, FEV_1 was analysed in the per protocol (PP) analysis in 13, 7, 8 and 8 patients, who had received placebo, 25 mg, 50 mg or 75 mg amitriptyline/day, respectively. After 14 days of treatment, the primary endpoint FEV_1 had improved significantly in the 25 mg/d amitriptyline group relative to placebo (FEV_1: +5.0% com-

pared to the placebo group; p = 0.048) (Table 3). No significant change of lung function was observed when patients took 50 mg and 75 mg of amitriptyline (Table 3).

In addition, the secondary endpoint ceramide was significantly reduced after 14 days of treatment with all amitriptyline doses relative to placebo (placebo: mean±SD: 2.0±1.55 relative fluorescence intensity; 25-75 mg amitriptyline: 0.92±0.67 relative fluorescence intensity; p = 0.042). All other secondary endpoints were not significantly different between the placebo group and the three amitriptyline treatment groups (p=0.37).

A carry over effect of FEV_1 on subsequent treatment courses was not detectable.

Amitriptyline was well received in the patient group and no SAEs were recorded at the end of the 28 day courses. From the 80 AEs, 35 were related or possibly related to the medication. Two well-known AEs of amitriptyline, i.e., xerostomia and tiredness [9], were significantly different between placebo and the three amitriptyline treatment groups (Table 4). Tiredness (2 patients), and obstruction (1 patient) were reported as causes of interruption of a single treatment course. Two patients described a reversible obstipation. One patient described stomach pain and increased perspiration in the night, which was later identified as under-dosage of pancreas enzymes. One patient (no. 10) described a pulse increase of 10 beats/min, leading to withdrawal from the study. Three patients described a transient vertigo, which did not require a change of medication. Dose reduction was reported in 12 treatment courses. Despite typical AEs of amitriptyline, 95% of the CF patients finished the study. We did not observe an increased number of respiratory tract infections in the amitriptyline-treated patient group indicating that the drug did not negatively affect the infection of the patients.

In summary, the study proves that amitriptyline is safe in CF patients and the lower doses are well tolerated. Furthermore, our data indicate that treatment with amitriptyline improves the lung function of CF-patients.

Discussion

Here we tested the hypothesis whether amitriptyline improves lung function in patients with CF. Since preliminary data from our pilot study indicated that amitriptyline therapy using 37.5 mg given twice daily for 14 days appears to be safe and efficacious, we subsequently performed a phase II trial. We confirmed that a lower dose of 25 mg once daily of amitriptyline increased FEV_1 relative to placebo in the PP analysis. A positive trend of amitriptyline efficacy was observed at day 14 in the patients who received 50 mg and 75 mg/d amitriptyline but the values did not reach significance most likely due to pulmonary infections caused by viral pathogens during the study period and, thus, a small study group. The positive effect of amitriptyline on lung function in the 25 mg CF treatment group is supported by the finding that ceramide levels determined in respiratory epithelial cells decreased significantly under amitriptyline therapy. This result clearly corroborates our initial observations from animal studies that treatment of CF mice with amitriptyline, a blocker of acid sphingomyelinase, prevented cell death and protected the animals from severe pulmonary *P. aeruginosa* infections [8].

The study group is too small and too heterogenous to perform a meaningful ITT analysis. In particular, the inclusion of all patients, even those with obvious pulmonary infections (during placebo or amitriptyline treatment) that had to be excluded according to the initial exclusion criteria of the study protocol, excludes an ITT analysis as the primary analysis method.

The present study also demonstrates that amitriptyline, a well established agent in treatment of major depression in adults [9], is safe at doses of 25 mg to 75 mg in all CF patients when given orally for a periode up to four weeks. No severe adverse drug reactions were reported in our pilot as well as the phase II study. As expected, typical AEs related to amitriptyline [9] such as xerostomia and tiredness were reported in the phase II study. In one case using 75 mg of amitriptyline dose reduction of 50% due to xerostomia and tiredness resulted in a well-tolerated use of amitriptyline. Although CF patients seem to have an increased/altered metabolism in general, the frequency and nature of side effects related to amitriptyline in those patients [11] did not differ from reported adverse drug reactions of the drug in non-CF patients. At a lower dose of amitriptyline using 25 mg/d, AEs were transitory and lasted only for a few days. This indicates that even CF patients, who are carriers of functionally relevant *CYP2D6* variants, may profit from a dose-adjusted amitriptyline therapy. In principle low doses of amitriptyline have been successfully administered for patients with major depression, fibromyalgia and irritable bowel syndrome [13] and, thus, such a treatment option might be also applied for CF patients. A further concern to use amitriptyline in CF patients, infected chronically with *P. aeruginosa* or other bacterial pathogens, is the fact that amitriptyline would 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 [12]. However, the number of acute exacerbations did not increase significantly in the amitriptyline treatment groups compared to controls demonstrating again the safety of the applied amitriptyline doses in the CF patient study group.

In summary, the present findings demonstrate the safety and the efficacy of treatment of CF patients with low doses of amitriptyline and justify to continue the development of amitriptyline as a new strategy to fight lung infections in patients with CF. It should be pointed out that an increase of 4-5% 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.

Acknowledgements

The pilot study was supported by DFG-grant Gu 335/16-1 to E.G. The Phase IIa trial was supported by a financial grant from Mukoviszidose e.V., Bonn, the German Cystic Fibrosis Association and the AKF-program of the University of Tübingen, Tübingen, Germany.

The authors thank Nadine Kemmler, Derek Zieker, Vanya Icheva, Desiree Schneider for measuring and analysing laboratory data, Wolfgang Brehm, Reinhard Lerch, Karsten Dick and Martin Stern for their role as subinvestigators and Reinhard Vonthein for part the data analysis.

References

1 Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, et al: Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989;245:1059-65.

2 Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al.: Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245:1066-73.

3 Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC: Identification of the cystic fibrosis gene: genetic analysis. Science 1989;245:1073-80.

4 Boucher RC: Airway surface dehydration in CF: pathogenesis and therapy. Annu Rev Med 2007;58:157-70.

5 CF Foundation, Patient Registry Annual Report 2004, Bethesda, Maryland, USA, 2005. http://www.cff.org/UploadedFiles/publications/files/2006

6 Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, Birrer P, Bellon G, Berger J, Weiss T, Botzenhart K, Yankaskas JR, Randell S, Boucher RC, Döring G: Reduced oxygen concentrations in airway mucus contribute to the early and late pathogenesis of Pseudomonas aeruginosa CF airways infection. J Clin Invest 2002;109:317-25.

7 Döring G, Hoiby N, for the Consensus Study Group: Early intervention and prevention of lung disease in cystic fibrosis: a European consensus. J Cyst Fibros 2004;3:67-91.

8 Teichgräber V, Ulrich M, Endlich N, Riethmüller J, Wilker B, De Oliveira-Munding CC, van Heeckeren AM, Barr ML, von Kürthy G, Schmid KW, Weller M, Tümmler B, Lang F, Grassme H, Döring G, Gulbins E: Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis. Nature Med 2008;14:382-91.

9 Hurwitz R, Ferlinz K, Sandhoff K: The tricyclic antidepressants desipramine causes proteolytic degradation of lysosomal sphingomyelinase in human fibroblasts. Biol. Chem. Hoppe Seyler 1994;375:447-50.

10 Kornhuber J, Tripal P, Reichel M, Terfloth L, Bleich S, Wiltfang J, Gulbins E: Identification of new functional inhibitors of acid sphingomyelinase using a structure-property-activity relation model. J. Med. Chem. 2008;51:219-37.

11 Parker AC, Pritchard P, Preston T, Smyth RL, Choonara I: Enhanced drug metabolism in young children with cystic fibrosis. Arch Dis Childhood 1997;77:239-41.

12 Grassmé H, Jendrossek V, Riehle A, von Kürthy G, Berger J, Schwarz H, Weller M, Kolesnick R, Gulbins E: Host defense against Pseudomonas aeruginosa requires ceramide-rich membrane rafts. Nature Med 2003;9:322-30.

13 Mörike K, Kivistö KT, Schaeffeler E, Jägle C, Igel S, Drescher S, Fux R, Marx C, Hofmann U, Engel C, Wagner F, Delabar U, Meisner C, Bail D, Böhm JO, Gleiter CH, Ziemer G, Rein JG, Hellberg KD, Eichelbaum M, Schwab M: Propafenone for the prevention of atrial tachyarrhythmias after cardiac surgery: a randomized, double-blind placebo-controlled trial. Clin Pharmacol Therapeut 2008;84:104-10.

14 Riethmueller J, Vonthein R, Borth-Bruhns T, Grassmé H, Eyrich M, Schilbach K, Stern M, Gulbins E: DNA quantification and fragmentation in sputum after inhalation of recombinant human deoxyribonuclease. Cell Physiol Biochem 2008;22:347-52.

15 Wittkowski KM, Lee E, Nussbaum R, Chamian FN, Krueger JG: Combining several ordinal measures in clinical studies. Stat Med 2004;23:1579-92

16 Senn S. Cross-over Trials. In: Clinical Research (Second Edition). Wiley 2003

17 Zanger UM, Raimundo S, Eichelbaum M: Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. Arch Pharmacol 2004;369:23-37.