Original Paper

Long-Term Pulmonal Therapy of Cystic Fibrosis-Patients with Amitriptyline

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Key Words

Cystic Fibrosis • Amitriptyline • Ceramide • CFTR

Abstract

Background/Aims: Several recent clinical studies revealed an accumulation of ceramide in bronchial epithelial cells of patients with cystic fibrosis (CF). Degradation of ceramide concentrations in lungs of CF patients employing the functional acid sphingomyelinase inhibitor amitriptyline revealed a benefit in lung function, weight and exacerbation rates.

Methods: To test for a beneficial effect of amitriptyline *in vivo*, we performed two phase II randomised, double-blind, placebo-controlled studies. CF patients were treated with 25 mg amitriptyline twice daily, i.e. a total dose of 50 mg/d. After those two studies part of the patients used amitriptyline in an off-label-use for routine treatment. These patients were observed after one, two and three years after continuous use of amitriptyline and were matched with those patients who were not treated. These patients were used as a control group. **Results:** After one year of treatment, forced expiratory volume in 1 sec predicted (FEV₁) increased significantly by 7.6±7.0%, p=<0.001, and weight increased by 2.1±2.3kg, p=<0.001 in the amitriptyline population (n=20), whereas FEV₁ decreased significantly in the control group by 1.8±3.3%, p=0.010, and weight increased by 1.1±2.7kg, p=0.010 (n=14). After two years of treatment, FEV₁ increased significantly by 5.6±10.3%, p=0.009, and weight increased by 3.6±2.9kg, p=<0.001 in the amitriptyline population (n=12). In contrast, FEV₁ decreased in the control group by 2.1±3.7%, p=0.051 and weight increased by only 0.4±2.9kg, p=0.31 (n=10). After three years of treatment, FEV₁ increased significantly by 7.7±8%, p=0.050, and weight increased by 7.3±3.8kg, p=0.016, in the amitriptyline population (n=5), whereas FEV₁ decreased in the control group by 1.0±1.3%, p=0.075 and weight increased by 0.4±1.5kg, p=0.29 (n=5). **Conclusion:** Amitriptyline significantly increases FEV₁, reduces ceramide in lung cells and increases weight of CF patients.

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder in western countries. The disease is 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 [4-8]. Chronic bacterial respiratory infections especially by *Ps. aeruginosa* have the greatest impact on morbidity and mortality of the patients [5]. Recent animal studies offer an attractive novel possibility to treat cystic fibrosis [6]. We demonstrated in mice genetically deficient for *Cftr* an accumulation of ceramide in tracheal and bronchial epithelial cells [9-11]. The results were independently confirmed by several studies and, most importantly, also transferred to the human situation demonstrating accumulation of ceramide in bronchial epithelial cells of patients with cystic fibrosis [12-18]. Ceramide mediates increased death of bronchial epithelial cells resulting in a release of dead cells and finally DNA into the bronchial lumen [9].

Ceramide is generated by de novo synthesis and by hydrolysis of sphingomyelin [19]. We have previously shown that genetic or pharmacological inhibition of the acid sphingomyelinase that converts sphingomyelin to ceramide in lysosomes, secretory lysosomes and on the plasma membrane normalizes the increased ceramide levels in cystic fibrosis mice [9, 18]. Pharmacological inhibition of the acid sphingomyelinase was achieved by the use of amitriptyline and other tricyclic antidepressants [9, 18]. Previous studies demonstrated that amitriptyline and structural similar compounds functionally inhibit the acid sphingomyelinase and thereby reduce cellular ceramide *in vitro* and well as *in vivo* and that the reduction of ceramide levels in the hippocampus of stressed mice by these compounds mediates the anti-depressive effects of the drugs *in vivo* [20-23].

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] and 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 [24].

These studies revealed that amitriptyline is safe in CF patients, since only mild adverse effects were observed [15, 24]. Moreover, the per protocol (PP) analysis of these studies demonstrated an increase of the forced expiratory volume in one second (FEV₁) after treatment with amitriptyline compared to the placebo group by 4.2 and 4.9% absolute to baseline after 4 weeks of treatment suggesting an improvement of lung functions by amitriptyline.

Here we performed a case control study on 20 CF-patients treated with 25 mg amitriptyline twice daily as an off-label-use for one or more years after previous participation in one of these trials mentioned above, controlled by 14 patients who did not use amitriptyline for the following years.

We demonstrated a marked increase of FEV₁ and body weight in the amitriptyline treatment group for one or more years. The side effects of the treatment are minor and amitriptyline is well tolerated by CF patients.

Materials and Methods

Study design

We performed in 2007 a phase IIa and in 2012 a phase IIb study in multicentric, randomised, double-blind, placebo-controlled, cohort study designs with 59 CF patients.

A case control study was initiated after these two studies were finished and part of the patients continued to take amitriptyline, which was possible in an off-label-use. 20 patients on amitriptyline treatment could be followed up for one, two and three years and 14 patients without amitriptyline treatment.

Patients

Patients older than 14 years were included in these studies based on the following criteria: cystic fibrosis was verified; 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. 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. During treatment period, patients were treated for 28 days.

After the follow up visit 20 out of 59 patients asked for continuing the amitriptyline treatment, which was done in an off-label-use (2 x 25 mg continuously for one to three years).

Outcome

The outcome parameter FEV₁ was defined as the best FEV₁ measured in one year (usually done 4 times or more per year) measured by spirometry (Jaeger, Höchberg, Germany). The outcome parameter body weight was also registered 4 times or more per year. The average of the weight measurements were taken for analysis. Adverse effects (AEs) were not found during the observation period. Pulmonary exacerbation rates defined after Fuchs et al. did not differ between treatment and control group and therefore no further analysis was performed.

FEV₁ and weight were compared between treatment and control group and determined one year before, just before and one, two and three years after the studies and calculated by student's t-test with significance level $\alpha=0.05$.

Results

From the 59 adolescent and adult CF patients, 20 patients received amitriptyline for one or more years and 14 patients could be analysed for one or more years without amitriptyline treatment under routine therapy conditions. The best of FEV₁ measurements in one year and the average of weight measurements per year were analysed.

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.

Table 1. Demographic data and baseline characteristics in cystic fibrosis patients treated with amitriptyline or not (controls). Presented are the characteristics of all included individuals, i.e. gender, age, weight, the forced expiratory volume in the 1st second (FEV₁), the percentage of patients with pancreas insufficiency, the amount of patients colonized with *P. aeruginosa*, *S. aureus*, multiple resistant *S. aureus*, *S. maltophilia*, Fungi like *A. fumigatus* or *Candida species*, *E. coli* and other, not further specified bacteria in the lung. ^aValues are means $\pm$ SD; MRSA: methicillin resistant *Staphylococcus aureus*

	Amitriptyline	Controls	p value
Characteristic	(n = 20)	(n = 14)	
Female Gender (%)	9	4	0.15
Age (years) ^a	26.7 $\pm$ 8.1	30.1 $\pm$ 10.3	0.14
Weight (kg) ^a	57.0 $\pm$ 13.4	60.7 $\pm$ 9.0	0.19
FEV ₁ % ^a	61.1 $\pm$ 16.9	65.8 $\pm$ 16.9	0.21
Pancreas insufficiency (%)	0	0	n.a.
<i>P. aeruginosa</i> , non mucoid	16	11	0.13
<i>P. aeruginosa</i> , mucoid	8	3	0.08
<i>St. aureus</i>	15	11	0.14
MRSA	1	0	n.a.
<i>Stenotrophomonas maltophilia</i>	1	1	n.a.
Fungi (<i>A. fumigatus</i> , <i>Candida spp</i>)	17	12	0.13
<i>E. coli</i>	3	2	0.19.
others	3	3	n.a.

After one, two and three years of amitriptyline treatment lung functions of CF patients, measured as FEV₁ predicted, improved significantly in the amitriptyline treatment group from a mean $\pm$ standard deviation (SD) 61.1 $\pm$ 16.9% to 68.7 $\pm$ 20.3%, $p=0.0001$ (difference of +7.6 $\pm$ 7.0%) in the one year group ($n=20$), from a mean $\pm$ SD of 59.5 $\pm$ 18.5% to 65.9 $\pm$ 21.7% (diff +6.5 $\pm$ 7.9%) in the first year and to 64.9 $\pm$ 22.2% (diff +5.6 $\pm$ 10.3%) in the second year, $p=0.009$ in the two year group ($n=12$), and from a mean $\pm$ SD of 56.8 $\pm$ 23.5% to 64.3 $\pm$ 26.1% (diff +7.6 $\pm$ 7.4%) in the first year to 64.4 $\pm$ 25.0% (diff +7.6 $\pm$ 7.4%) in the second year and to 64.5 $\pm$ 25.7% (diff +7.7 $\pm$ 8.0%) in the third year, $p=0.05$ in the three year group ($n=5$). In marked contrast, the control group FEV₁ predicted consistently decreased by 1.0 to 2.6% (Table 2, Figure 1).

Table 2. FEV₁ efficacy [% predicted] of amitriptyline in patients with cystic fibrosis at baseline, the year before, after one year, after two and three years of amitriptyline treatment compared to controls. Lung function was determined in the amitriptyline and the control group as forced expiratory volume in one second (FEV₁). The number (n) of patients in the different groups is given. Values represent geometric means $\pm$ standard deviations. Significant difference of FEV₁ between placebo and amitriptyline-treated groups are calculated by Student's t-test.^{1,2} Differences between the year before to baseline and baseline to year 1 after treatment are given.^{3,4} Differences between baseline to year 2 after treatment and between baseline to year 3 after treatment are given. *values represent means $\pm$ SD. Student's t-test was used

Treatment	Number of patients	Year before	Baseline before amitriptyline studies	Year 1 after	Year 2 after	Year 3 after	p value
Amitriptyline	20	61.5 $\pm$ 18.5	61.1 $\pm$ 16.9	68.7 $\pm$ 20.3	-	-	0.0001
		Differences ^{1,2}	-0.5 $\pm$ 4.4	7.6 $\pm$ 7.0	-	-	0.0008
	12	57.3 $\pm$ 18.6	59.5 $\pm$ 18.5	65.9 $\pm$ 21.7	64.9 $\pm$ 22.2	-	0.009
		Diff.s	2.2 $\pm$ 2.4	6.5 $\pm$ 7.9	5.6 $\pm$ 10.3	-	0.075
	5	55.3 $\pm$ 23.8	56.8 $\pm$ 23.5	64.3 $\pm$ 26.1	64.4 $\pm$ 25.0	64.5 $\pm$ 25.7	0.050
		Diff.s ^{1,2,3,4}	1.5 $\pm$ 2.3	7.6 $\pm$ 7.4	7.6 $\pm$ 7.4	7.7 $\pm$ 8.0	0.07
Controls	14	70.0 $\pm$ 17.4	65.8 $\pm$ 24.0	65.6 $\pm$ 17.8	-	-	0.010
		Diff.s	-2.6 $\pm$ 5.1	-1.8 $\pm$ 3.3	-	-	0.32
	10	65.5 $\pm$ 16.0	63.7 $\pm$ 17.0	61.8 $\pm$ 16.5	61.6 $\pm$ 16.9	-	0.051
		Diff.s	-1.8 $\pm$ 5.9	-1.9 $\pm$ 3.0	-2.1 $\pm$ 3.7	-	0.45
	5	64.3 $\pm$ 20.0	63.1 $\pm$ 20.4	62.1 $\pm$ 19.1	61.7 $\pm$ 22.5	62.1 $\pm$ 21.1	0.075
		Diff.s	-1.2 $\pm$ 7.0	-1.0 $\pm$ 3.9	-1.4 $\pm$ 3.0	-1.0 $\pm$ 1.3	0.48

Fig. 1. Differences of FEV₁ predicted in % in long term with amitriptyline treated cystic fibrosis patients compared to controls. Left graph: 20, 12 and 5 CF patients with differences of FEV₁ after 1, 2 and 3 years of treatment with amitriptyline; means $\pm$ SD are given. Right graph: 14, 10 and 5 CF patients with differences of FEV₁ after 1, 2 and 3 years without amitriptyline treatment; means $\pm$ SD are given.

After one to three years of amitriptyline treatment weight in kg of CF patients improved significantly in the amitriptyline treatment group from a mean $\pm$ SD of 57.0 ± 13.4 kg to 59.2 ± 13.1 kg, $p=0.0002$ (diff $+2.1 \pm 2.3$ kg) in the one year group, from a mean $\pm$ SD of 57.2 ± 16.1 kg to 59.4 ± 16.2 kg (diff $+2.2 \pm 1.6$ kg) in the first year and to 60.8 ± 16.8 kg (diff $+3.6 \pm 2.9$ kg) in the second year, $p=0.0006$, in the two year group and from a mean $\pm$ SD of 71.5 ± 21.2 kg to 74.8 ± 20.8 kg (diff $+3.3 \pm 1.5$ kg) in the first year, to 76.8 ± 21.9 kg (diff $+5.3 \pm 3.3$ kg) in the second year and to 78.8 ± 23.0 kg (diff $+7.3 \pm 3.8$ kg) in the third year, $p=0.0028$, in the three year group. Body weight of the control group weight remained unchanged by -0.1 to 1.1 kg (Table 3, Figure 2).

Table 3. Weight gain [kg] after amitriptyline in patients with cystic fibrosis at baseline, the year before, after one year, after two and three years of amitriptyline treatment compared to controls. Weight was determined in the amitriptyline and the control group. The number (n) of patients in the different groups is given. Values represent means $\pm$ standard deviations. Significant difference of weight between placebo and amitriptyline-treated groups are calculated by Student's t-test. ^{1,2} Differences between the year before to baseline and baseline to year 1 after treatment are given. ^{3,4} Differences between baseline to year 2 after treatment and between baseline to year 3 after treatment are given. *values represent means $\pm$ SD. Student's t-test was used

Treatment	Number of patients	Year before	Baseline before amitriptyline studies	Year 1 after	Year 2 after	Year 3 after	p value
Amitriptyline	20	56.7 ± 13.4	57.0 ± 13.4	59.2 ± 13.1	-	-	0.0002
		Differences ^{1,2}	0.3 ± 0.8	2.1 ± 2.3	-	-	0.00085
	12	57.0 ± 15.8	57.2 ± 16.1	59.4 ± 16.2	60.8 ± 16.8	-	0.0006
		Diff.s	0.1 ± 0.8	2.2 ± 1.6	3.6 ± 2.9		0.0028
	5	71.5 ± 20.4	71.5 ± 21.2	74.8 ± 20.8	76.8 ± 21.9	78.8 ± 23.0	0.016
		Diff.s ^{1,2,3,4}	1.1 ± 0.8	3.3 ± 1.5	5.3 ± 3.3	7.3 ± 3.8	0.069
Controls	14	60.0 ± 9.1	60.7 ± 9.0	61.8 ± 9.6	-	-	0.010
		Diff.s	0.6 ± 2.4	1.1 ± 2.7			0.26
	10	57.3 ± 4.1	58.1 ± 4.6	58.8 ± 5.2	58.5 ± 6.0	-	0.31
		Diff.s	0.8 ± 2.9	0.7 ± 2.1	0.4 ± 2.9		0.33
	5	58.2 ± 5.2	58.8 ± 5.9	59.3 ± 6.6	58.7 ± 7.7	59.2 ± 5.8	0.29
		Diff.s	0.6 ± 3.1	0.5 ± 2.3	-0.1 ± 2.2	0.4 ± 1.9	0.46

Fig. 2. Differences of weight in kg in long term with amitriptyline treated cystic fibrosis patients compared to controls. Left graph: 20, 12 and 5 CF patients with differences of weight after 1, 2 and 3 three years of treatment with amitriptyline; means $\pm$ SD are given. Right graph: 14, 10 and 5 CF patients with differences of weight after 1, 2 and 3 three years without amitriptyline treatment; means $\pm$ SD are given.

Amitriptyline was well-received in years of treatment and no severe adverse effect, e.g. like inpatient treatment was observed in the amitriptyline-treated group. None of other typical adverse effects of amitriptyline, i.e., vertigo, arterial hypotonia, long QT syndrome, tremor, dyskinesia, obstipation, dysuria or glaucoma were observed in our observational study, very likely because of the low dose administered.

Pulmonary exacerbation rates were rare and did not differ between both groups. It should be mentioned that exacerbation rates are in general low in Europe, because of routine treatment with intravenous antibiotics.

Taken together, our data demonstrate a positive trend of amitriptyline to increase lung function and weight in long-term treatment in CF patients in contrast to the natural decrease of lung function over years in non-treated patients.

Discussion

In the present observational study, we tested whether amitriptyline improves lung functions and weight in patients with CF in a long-term setting. Since data from our phase IIa and IIb studies [15, 24] indicated that amitriptyline therapy using 25 mg given twice daily is safe and increases FEV₁, we were able to long-term follow patients who received amitriptyline in a routine setting as an off-label use.

The observational study demonstrates a significant increase of lung function and weight of CF-patients over years while being treated with amitriptyline, whereas the control group declined in lung function by 1-2% per year, consistent to previous and well-known findings [25]. Although our short-term studies showed that amitriptyline reduces ceramide levels in airway epithelial cells of CF patients [15, 24], further long-term and phase III studies are required to quantify ceramide and other sphingolipid-biomarkers in sputum and respiratory epithelial cells to link a decrease of ceramide with the observed effect amitriptyline on lung functions.

Interestingly, CF patients also moderately gained weight in the present long-term study. This could be an effect of amitriptyline on intestinal epithelial cells and, thus, improved nutrition, but it could be also a secondary effect of the improved lung function. Further animal studies are required to define the role of ceramide and sphingolipids in the gut of CF animals and patients.

The study also demonstrates that amitriptyline, a well-established agent in treatment of major depression in adults [26], is safe at a dose of 2 x 25 mg/day in all CF patients when continuously given orally for years. No severe adverse drug reactions were reported, while typical AEs related to amitriptyline such as xerostomia and tiredness disappeared after a few weeks after initiation treatment.

We have previously shown that ceramide is not only harmful in the respiratory tract, but that a complete lack of ceramide release in acid sphingomyelinase-deficient mice also sensitizes these animals to bacterial infections [9, 27, 28]. This is most likely caused by a defect of a release of reactive oxygen species in cells lacking the acid sphingomyelinase [28]. However, this phenotype requires more than 90% reduction of the acid sphingomyelinase expression/activity. It is impossible to achieve such an inhibition with amitriptyline, since that drug competes with the acid sphingomyelinase for binding to the inner lysosomal membrane [9, 23]. Therefore the maximum inhibition of the acid sphingomyelinase induced by amitriptyline is 50-60% resulting in a very safe use of the drug [9, 23]. This partial reduction of the acid sphingomyelinase results in a normalization of the increased ceramide-levels in cystic fibrosis lungs, but not in a reduction of ceramide levels and therefore the drug does not impair the local response to *P. aeruginosa* [9]. Accordingly, the number of acute pulmonary exacerbations did not increase significantly in the amitriptyline group compared to controls demonstrating the safety of the applied amitriptyline dose in the CF patient study group.

Ceramide in bronchial epithelial cells has been shown to trigger cell death and thereby to mediate a release of DNA into the airways, which greatly facilitates infections with

P. aeruginosa and also impairs the mucociliary clearance [9, 18]. In addition, ceramide triggers, via still unknown mechanisms, the formation and release of pro-inflammatory mediators and thereby pulmonary inflammation [9]. Normalization of pulmonary ceramide concentrations by amitriptyline also normalizes these pathologies [9, 18]. 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 efficacy and safety 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. It should be pointed out that an increase of 5-8% of lung function (FEV₁) is comparable to the beneficial effects of other drugs (inhalative antibiotics, secretolytics) commonly used to treat cystic fibrosis, especially CFTR modulators and correctors like Orkambi®. However, amitriptyline can be used in all CF patients regardless of the genetic defect, it is a well-known, long-term used drug with a very good safety profile at the low dose used in the study and it may also have very beneficial, systemic effects in cystic fibrosis patients, for instance in the gut.

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Disclosure Statement

We declare that we have no conflict of interest.

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