



Original Article

A randomized placebo-controlled trial of miglustat in cystic fibrosis based on nasal potential difference[☆]

Anissa Leonard^{a,*}, Patrick Lebecque^a, Jasper Dingemans^b, Teresinha Leal^c^a Pediatric Pulmonology & Cystic Fibrosis Unit, Cliniques St Luc, Université catholique de Louvain, 10 Avenue Hippocrate, 1200 Brussels, Belgium^b Actelion Pharmaceuticals Ltd, Gewerbstrasse 16, CH-4123 Allschwil, Switzerland^c Louvain Center for Toxicology and Applied Pharmacology, Cliniques Universitaires St Luc, Université catholique de Louvain, 10 Avenue Hippocrate, 1200 Brussels, Belgium

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Abstract

Background: Preclinical data suggest that miglustat could restore the function of the cystic fibrosis transmembrane conductance regulator gene in cystic fibrosis cells.

Methods: Single-center, randomized, double-blind, placebo-controlled, crossover Phase II study in 11 patients (mean±SD age, 26.3±7.7 years) homozygous for the F508del mutation received oral miglustat 200 mg t.i.d. or placebo for two 8-day cycles separated by a 14-day washout period. The primary endpoint was the change in total chloride secretion (TCS) assessed by nasal potential difference.

Results: No statistically significant changes in TCS, sweat chloride values or FEV₁ were detected. Pharmacokinetic and safety were similar to those observed in patients with other diseases exposed to miglustat.

Conclusions: There was no evidence of a treatment effect on any nasal potential difference variable. Further studies with miglustat need to adequately address criteria for assessment of nasal potential difference.

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Keywords: Miglustat; Cystic fibrosis; F508del; Efficacy; Tolerability; Pharmacokinetics

1. Introduction

Cystic fibrosis (CF) is the most common severe genetic disease in Caucasian populations. It is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene [1]. The encoded protein mainly acts as a cAMP-dependent chloride channel, but also regulates other membrane transporters such as the epithelial sodium channel ENaC [2]. Ion transport dysfunction in CF reduces the airway surface liquid volume and results in repeated chronic infections and an exaggerated inflammatory response.

More than 1700 mutations of the *CFTR* gene have been identified. Approximately 70% of CF patients are homozygous for the F508del mutation. The mutant protein resulting from the F508del *CFTR* gene mutation is recognized as misfolded, becomes retained by the endoplasmic reticulum (ER) quality control, and is subsequently degraded within the ubiquitin-proteasomal pathway (UPP) [3].

Miglustat (N-butyldeoxynojirimicin, Zavesca®) is an iminosugar currently marketed for adult patients with type 1 Gaucher disease for whom enzyme replacement therapy is unsuitable/not a therapeutic option and for children and adults with Niemann-Pick type C disease [4,5]. This compound is an inhibitor of several enzymes, including α -glucosidase of the ER. Recent in vitro studies showed that miglustat is able to correct impaired trafficking of F508del-*CFTR* protein [3,6,7]. Moreover, in vivo studies in F508del transgenic mice provided evidence that nasal delivery of miglustat normalizes sodium transport and *CFTR*-dependent chloride transport [8].

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* Corresponding author. Tel.: +32 2 7641939; fax: +32 2 7648906.

E-mail address: anissa.leonard@uclouvain.be (A. Leonard).

It has been proposed that miglustat may interfere with the activity of calnexin, an important chaperone protein, on the intracellular processing of F508del-CFTR protein [6]. Before it interacts with calnexin, the F508del-CFTR protein must be glycosylated, and miglustat may block this step due to its inhibitory effect on ER α -glucosidase, thus preventing UPP-mediated degradation of the misfolded protein [3].

This proof-of-concept study was designed to test the hypothesis that miglustat may restore CFTR function in CF patients homozygous for the F508del mutation.

2. Materials and methods

This was a single-center, double-blind, randomized, placebo-controlled, two-period/two-treatment crossover, Phase IIa proof-of-concept study. The study protocol was approved by the local Ethics Committee, and study procedures were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Informed written consent was obtained from all patients and parents (when appropriate).

2.1. Patients

Patients enrolled in the study had a confirmed diagnosis of CF as established by typical clinical features and abnormal sweat test findings (sweat chloride >60 mmol/L). They were aged 12 years or older, had clinically stable lung disease, and carried the F508del mutation on both alleles of the CFTR gene. Other criteria for eligibility included a forced expiratory volume in 1 s (FEV₁) of at least 25% of the predicted value [9] and a blood oxygen saturation of at least 88% in room air. Exclusion criteria included: any acute respiratory infection within 2 weeks of screening; severe renal impairment; history of neuropathy or clinically significant lactose intolerance; active or passive smoking as assessed by exhaled CO measurement (Smokelyzer®); pregnancy; breastfeeding and lack of effective contraception in women.

2.2. Study design

Patients were randomized to one of two treatment sequences: placebo/miglustat or miglustat/placebo. Miglustat or matching placebo was administered orally at a dose of 200 mg t.i.d. for

8 days. The two treatment periods were separated by a washout period of at least 14 days (Fig. 1). There were no restrictions regarding concomitant medications, except that other investigational drugs and/or therapies (e.g., gene therapy) were not allowed.

2.3. Endpoints

The primary pharmacodynamic endpoint was the change in total chloride secretion (TCS) from baseline (pre-dose on Day 1) to the end-of-treatment (Day 8) of each treatment period. TCS was quantified as the sum of nasal potential difference (NPD) responses during consecutive perfusions of the nasal mucosa with a chloride-free buffer with amiloride and a chloride-free buffer with amiloride and isoproterenol. Response of NPD to treatment was predefined as an on-treatment improvement in TCS of at least -5.0 mV from baseline, based on previously published data [10].

Secondary endpoints included changes from baseline to the end of each treatment period in: 1) basal NPD response; 2) NPD response to a perfusion with amiloride; 3) NPD response to a perfusion with a chloride-free solution with amiloride; 4) NPD response to a perfusion with a chloride-free solution with amiloride, isoproterenol, and ATP; 5) sweat chloride concentration (sweat production stimulated by pilocarpine iontophoresis). The safety and tolerability of miglustat in patients with CF were assessed by adverse event monitoring, hematology, clinical chemistry, electrocardiograms (ECGs), vital signs, and physical examinations.

Miglustat pharmacokinetics were also evaluated, and changes in FEV₁ were measured as an exploratory endpoint.

2.4. Measurement of NPD

Transepithelial NPD was measured according to a previously published method [11]. Briefly, NPD values were recorded using a data memory high impedance voltmeter (Knick Portamess®, Elektronische Messgeräte, Berlin, Germany) connected to an Ag/AgCl exploring electrode built into a modified Foley urinary catheter, which was inserted along the floor of the nose (4–7 cm from the inferior turbinate). Only one nostril was evaluated. A reference electrode was taped to a lightly abraded skin area on the forearm.

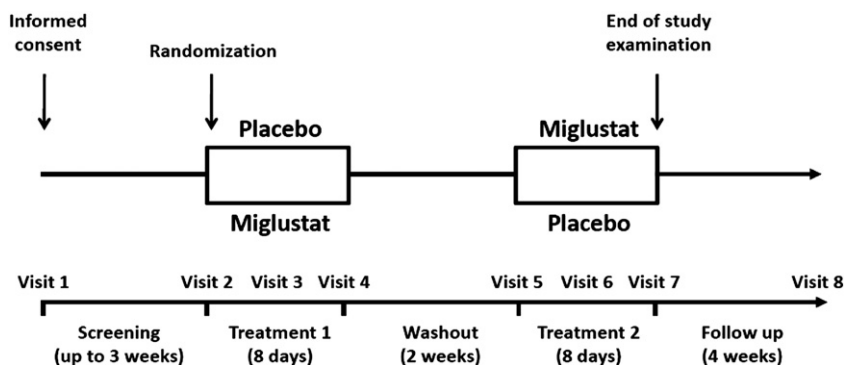


Fig. 1. Study design.

Exploring electrode bridges were filled with ECG cream (Signa cream/Ringer's lactate; 1:1 v/v). The nasal catheter was fixed at the site of the most stable negative voltage (PD_{max}) and a phosphate-buffered Ringer's solution was perfused for 1 min. Subsequent, sequential perfusions were performed with: 1) a phosphate-buffered Ringer solution and 100 μM amiloride; 2) a chloride-free Ringer solution and 100 μM amiloride; 3) a chloride-free Ringer solution with 100 μM amiloride and 10 μM isoproterenol; 4) a chloride-free Ringer solution with 100 μM amiloride, 10 μM isoproterenol, and 100 μM ATP. All solutions were warmed to 36.9 °C. Prior to any NPD assessment, the operator ensured that patients did not present any sign of upper airway infection within 2 weeks before the study, and that the concentration of exhaled carbon monoxide did not exceed 25 ppm.

NPD values were recorded once a stable negative voltage value had been reached, which usually took approximately 5 min. Recorded data were analyzed using Paraly SW 105[®] computer software. Separate, blinded assessments of tracings were conducted at the end of the study by TL.

2.5. Bioanalytical and pharmacokinetic analyses

Trough miglustat plasma concentrations were collected on Day 4 of both treatment periods. On Day 8, blood samples were withdrawn just prior to the morning dose of study medication and at 1, 2.5, 4, 6, and 8 h after dosing. Plasma concentrations of miglustat were determined using a validated LC-MS/MS assay as recently described [12]. The calibration range was 10–10,000 ng/mL, the coefficient of variation was <13.5%, and assay inaccuracy was <6.4%.

Pharmacokinetic parameters were determined by model-independent methods (WinNonlin Professional Version 4.0.1; Pharsight, Mountain View, California, USA) whereby AUC (area under the plasma concentration–time curve) was calculated using the linear trapezoidal method.

2.6. Statistics

Based on historical data obtained from healthy controls, a sample size of 11 patients was predicted to have 90% power to detect a difference in TCS of –5.0 mV, assuming a standard deviation of 4.4 mV, using a paired *t*-test with a 0.05 two-sided significance level [13]. Potential carry-over and period effects were evaluated using mixed models. A two-sided paired *t*-test was used to evaluate the null hypothesis that there would be no difference between the effects of miglustat and placebo on NPD; –5.0 mV was set as the required miglustat vs. placebo difference to qualify the alternative hypothesis that the effects of the two treatments differed. Statistical analyses of secondary endpoints based on paired, two-sided treatment differences were exploratory in nature.

3. Results

Eleven patients (mean±SD age, 26.4±7.7 years; six male, five female) were enrolled, all of whom completed the study.

Characteristics of the study group at the beginning of each treatment period were similar (Table 1).

3.1. NPD

There was no evidence of a treatment effect on any NPD variable. Unexpectedly, TCS was equal to or less than –5 mV at baseline in 6/11 patients during the miglustat treatment period and in 7/11 patients during the placebo period. There was a mean±SD increase of 0.55±4.56 mV (95% CL –2.51, 3.60) in TCS response from baseline to Day 8 with miglustat treatment, and a mean decrease of –0.09±3.83 mV (95% CL –2.67, 2.48) with placebo. The paired miglustat vs. placebo difference in TCS was 0.64±6.25 mV (95% CL –3.56, 4.83; *p*=0.74) [unadjusted *t*-test]. The paired difference of chloride secretion between the two baseline tests was –0.5±2.6 mV, indicating good within-subject reproducibility.

Table 2 shows changes in all pharmacodynamic endpoints from baseline for each treatment period. No significant changes from baseline were observed in any of these endpoints. Baseline values obtained at the beginning of the miglustat and the placebo period were not different (*p*=0.25; data not shown).

3.2. Sweat chloride

As shown in Table 2 miglustat treatment did not modify sweat electrolytes and intra-subject variability of sweat chloride values was low during repeated testing in this study, with a mean coefficient of variation of 5%.

3.3. Pharmacokinetics

A total of 88 plasma samples were analyzed. Miglustat plasma concentrations had reached steady-state conditions by Day 4. On Day 8, miglustat pharmacokinetics was characterized by a *t*_{max} of 2.5 h, a *C*_{max} of 2.5 μg/mL, and an AUC of 14 μg.h/mL. The mean plasma concentration–time profile is shown in Fig. 2.

Table 1

Demographics and characteristics of all 11 patients at the beginning of each treatment period.

	First period	Second period
Age (y)	26.4±7.70	
Gender (n)		
Male	6	
Female	5	
Weight (kg)	64.1 9.6	63.3±10.3
FEV ₁ (% predicted)	80.7±18.7	77.8±21.3
Sweat chloride concentration (mmol/L)	111.9±11.8	110.6±10.8
Nasal potential difference		
Basal PD (mV)	–43.2±7.2	–43.7±7.1
Total chloride secretion (mV)	–5.8±3.8	–4.7±3.4

Data are presented as mean (±SD) except gender (n).

Table 2
Changes from baseline in primary and secondary pharmacodynamic endpoints at the end of the two treatment periods (all patients; n=11).

	Miglustat period	Placebo period	p-value
Primary endpoint			
Total chloride secretion (mV)	-1.4±4.3	0.8±3.2	0.26
Secondary/exploratory endpoints			
Basal NPD (mV)	2.2±7.0	-1.5±6.5	0.18
Amiloride response (mV)	-1.0±10.5	-2.0±8.3	0.73
Sweat chloride concentration (mmol/L)	-1.9±9.0	-1.5±8.4	0.97
FEV ₁ (% predicted)	-1.4±4.6	-2.3±4.0	0.64

Data presented as mean±SD; p-values are from paired t-test (miglustat vs. placebo).

3.4. Adverse events

Adverse events were of mild or moderate intensity and no patient discontinued study drug during the study. All patients experienced at least one adverse event during miglustat treatment and 6/11 experienced adverse events on placebo. The most frequent adverse event was diarrhea, occurring in 10/11 patients (91%) on miglustat treatment and 4/11 patients (36%) on placebo treatment (Table 3). There were no clinically significant findings in relation to laboratory variables or ECG abnormalities. There were no deaths or SAEs reported during the study. No clinically relevant changes were observed in vital signs, except in one patient who experienced worsening of pre-existing hypertension during miglustat treatment.

4. Discussion

This is only the second placebo-controlled trial conducted in the past decade to investigate the possible improvement of F508del-CFTR function using a pharmacological agent [14]. In this study, no evidence was obtained of a treatment effect

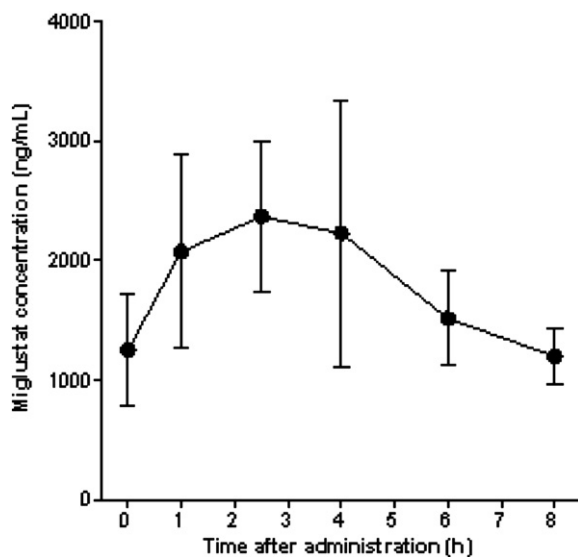


Fig. 2. Plasma concentration–time profile of miglustat on Day 8 in cystic fibrosis patients treated with miglustat 200 mg t.i.d. (all patients; n=11). Data points represent mean (±SD).

Table 3
Summary of reported adverse events.

	Miglustat	Placebo
Total patients with at least one AE	11 (100%)	6 (54.5%)
Diarrhea	10 (91%)	4 (36%)
Abdominal discomfort	3 (27%)	–
Headache	3 (27%)	–
Abdominal pain	2 (18%)	1 (9%)
Aphthous stomatitis	1 (9%)	–
Chills	1 (9%)	–
Dizziness	1 (9%)	–
Hypertension	1 (9%)	–
Insomnia	–	1 (9%)
Decreased appetite	–	1 (9%)

on any NPD variable. However, NPD findings from this study are difficult to interpret due to poor intra-individual repeatability of TCS measurements and higher than expected baseline values for TCS, offering little or no room for improvement.

NPD is the only in vivo functional surrogate marker of CFTR function at the level of the human respiratory tract, and TCS is a key disease variable as it primarily reflects CFTR chloride channel activity. Accordingly, all published early-phase clinical trials of medications aimed at restoring mutant CFTR function have postulated that efficacy should be demonstrated by a significant shift in TCS towards the normal range; ten studies of this sort have been published over the past 11 years [10,15–23]. Still, although physiologically meaningful, NPD is not a very robust efficacy index and is prone to error.

The range of usual TCS values in CF patients is narrow (roughly -10 mV to +10 mV) and mean TCS is close to zero, precluding calculation of a coefficient of variation for the parameter. Further, the within-subject variability of TCS has been shown to be larger than its between-subject variability, which indicates low repeatability [24]. Data concerning this variability are limited, have been obtained using different setups with subtle technical differences, and have most often been derived from comparisons of tracings obtained in two nostrils during a single session. Operator skill, a number of technical details, the influence of modifier genes, and the effects of concurrent medications have all been identified as potential sources of NPD variability during multicenter clinical trials [24]. Our NPD measurements were performed by one skilled operator, which should have lowered within-subject variability. However, our values were recorded in a single nostril, while averaging measurements from two nostrils could have reduced variability [18]. In addition, the most recent equipment for measuring NPD was not available at the time of the study; newer equipment has been shown to improve NPD tracing stability and to reduce artifact frequency by 75% [25].

NPD measurements have long been used in our center for diagnostic purposes. Reference values and cut-offs of -30 mV for PDmax and -10 mV for TCS were established in 2002 [11,26]. Such values are identical to those recently suggested by Middleton and House [27]. However, it should be noted that in clinical trials, a significant response of TCS to treatment is usually defined as a change of at least -5 mV [19].

In this study, mean baseline PDmax values for the miglustat treatment period and the placebo treatment period fell within the CF range and were identical to those recently reported in 76 patients homozygous for the F508del mutation (-43 mV) [27]. The mean coefficient of variation for PDmax was 16%.

The most puzzling finding in this study was the unexpected residual chloride secretion observed at baseline in half of the enrolled patients. Three out of 22 baseline measurements yielded TCS ≤ -10 mV, and a further six yielded values between -6 mV and -9 mV. This was not expected as published TCS values in classic CF patients are typically around 0; means range from 4 mV [28] to -3 mV in our center [29]. It is likely that some methodological factor(s) contributed to this range. For instance, the use of agar catheters shifted TCS in CF patients to less positive values, with a mean difference of 2 mV as compared with the perfusion method [25]. So-called ‘chloride secretors’ with TCS values more negative than -5 mV have been reported in approximately 10% of cases by several skilled operators investigating groups of subjects, completely or mostly homozygous for the F508del mutation, [27,29]. In one multicenter study, 15/75 (20%) F508del homozygous patients were categorized as chloride secretors, with similar proportions observed across three hospitals [29].

Methodological factors and poor reproducibility of TCS measurements are also likely involved to some extent, and repeating measurements using the most recent setup and protocol could help in this respect. Environmental factors such as concomitant medications could also be involved. Several symptomatic medications often prescribed in CF might influence ion transport across the nasal epithelia, but this is seldom evaluated. In the present study, azithromycin, inhaled salmeterol, inhaled acetylcysteine, and nasal steroids were prescribed to 10, 11, 4, and 5 out of the 11 enrolled patients, respectively.

Fulfillment of inclusion criteria in this study did not confirm the absence of bias. As do most investigators, we chose to select reliable candidates in order to minimize patient dropout. A compassionate bias may also have led us to focus on patients who were in relatively good health to avoid subjecting more ill patients to a demanding protocol. As a result, the study group included young adults with high FEV₁. Furthermore, when assessed by linear regression using the best value of each quarter, the mean annual decline in FEV₁ over the past 10 years was -1.2% (i.e., patients improved over time). Even considering the mean decline of -0.4% over 8 years recently reported in this clinic [30], it would appear that the patients enrolled were doing especially well.

Although miglustat treatment did not modify sweat electrolytes this finding does not preclude the effectiveness of the drug because, at this point in time, the extent to which an agent capable of rescuing chloride secretion by respiratory epithelial cells also influences absorptive sweat duct epithelium remains unclear.

The pharmacokinetics and tolerability of miglustat in this cohort of CF patients were similar to those observed in patients with other conditions [4]. These findings are of potential importance, given that the pharmacokinetics of a number of drugs are altered in patients with CF, and that patients with pancreatic insufficiency are especially prone to gastrointestinal disturbances.

In conclusion, no miglustat effect was detected in the present study. A further, larger study of oral miglustat in CF patients homozygous for the F508del mutation that incorporates the current NPD setup and a standardized protocol would help to clarify possible technical limitations of the NPD test as it was performed. Pre-enrolment screening of NPD measurements would be recommended in order to exclude chloride secretors, and caution should be taken to avoid selection biases that might exclude less healthy patients. Blinded over-reading, employing predefined criteria for low-quality tracings would be recommended to allow exclusion of data, if necessary. In addition, in light of recent data a longer duration of miglustat treatment and possible combination therapy with a potentiator compound could be considered [31].

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- PL and TL participated in the design of the study; AL, JD, PL, and TL in the collection, analysis, and interpretation of data; JD in the writing of the study report; AL, JD, PL, and TL in the manuscript.
- PL has received consulting honoraria; TL research grant funds; AL, PL, and TL travel expenses from Actelion Pharmaceuticals Ltd.

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