Association of CFTR activity in sweat test, NPD, and ICM with ivacaftor and lumacaftor serum levels in Phe508del homozygous patients with cystic fibrosis

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Abstract

Combination therapy with the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) corrector lumacaftor and the CFTR potentiator ivacaftor has demonstrated significant impact on clinical parameters in Phe508del homozygous people with CF. Whether these changes under treatment are correlated to serum levels of both drugs had yet to be investigated. We therefore analyzed data from our previous study (OrkambiFacts, ClinicalTrials.gov Identifier: NCT02807415). In summary, we did not find statistically significant correlations between serum drug levels and changes in clinical parameters and biomarkers of CFTR function such as FEV1, BMI, sweat chloride, nasal potential difference (NPD) and intestinal current measurement (ICM). Absolute blood levels of lumacaftor or ivacaftor do not seem to be informative biomarkers to predict clinical improvement or the attenuation of the basic defect.

Introduction

Combination therapy with the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) corrector lumacaftor and the CFTR potentiator ivacaftor has demonstrated reduction of pulmonary exacerbations and a gain of body weight and lung function in Phe508del homozygous people with CF [1, 2]. We could previously show that lumacaftor-ivacaftor improves CFTR function determined by nasal potential difference (NPD) and intestinal current measurement (ICM) to 10 to 20% of normal CFTR activity. This effect on CFTR function was observed even in the absence of short term effects on lung function [9] Whilst the effects of lumacaftor/ivacaftor therapy on numerous facets of basic defect and clinical disease have been reported [3], published information about the pharmacokinetics of the drugs, the association of drug concentration in tissues and body fluids with clinical outcome and the relationship between serum concentrations and the effect on CFTR function remain scarce [4- 7, 12].

Lumacaftor and ivacaftor are subject to clinically relevant drug-drug interactions between the CFTR modulators themselves and other concomitant medications used by people with CF. Lumacaftor is a CYP3A4/2C8/2C9/2C19 inducer and thus influences the turnover of ivacaftor, a CYP3A4 substrate [8]. Moreover, the two CFTR modulators are highly hydrophobic compounds. Consequently the tissue distribution of the drugs will depend on the body composition of fat, muscles and water which is highly variable

among CF patients depending on the extent of malabsorption and its nutritional management and physical activity.

In a prospective observational study we have examined clinical outcomes and biomarkers of CFTR function in Phe508del homozygous CF patients before and 8-16 weeks after initiation of lumacaftor-ivacaftor therapy [9]. Here we report on the association of serum concentrations of lumacaftor and ivacaftor with biomarkers of CFTR function and clinical outcomes obtained on the same day.Here we report on the association of serum concentrations of lumacaftor and ivacaftor with biomarkers of CFTR function and clinical outcomes obtained on the same day.

Materials and Methods

This prospective observational post-approval multicenter study (ClinicalTrials.gov Identifier: NCT02807415) was conducted at three CF centers of the German Center for Lung Research (DZL) in Phe508del homozygous CF patients aged 12 years and older. Prior to and 8 – 16 weeks after the initiation of lumacaftor-ivacaftor therapy anthropometry, lung function, sweat chloride concentrations, NPD and ICM were measured in all participants [9, 11]. Collection of blood and rectal biopsies for ICM, sweat test and NPD measurements were performed within 2 to 3 hours after the administration of the morning dose of lumacaftor/ivacaftor. Serum samples were stored at -30°C until further analysis.

Serum levels of lumacaftor and ivacaftor were analyzed by a validated liquid chromatography method with UV and mass detection. Briefly, 200 µl serum was deproteinised by the addition of 600 µl methanol and subsequent centrifugation at 13,000 rpm for 15 min. The supernatant was separated by an UltiMate 3000 UHPLC system (Thermo, Waltham, Ma) on a Reprosil Pur Basic C18 (3µm, 100x2mm) column with 0.1% formic acid (v/v) / methanol as mobile phase. Lumacaftor was quantified by UV-spectrophotometry at 254 nm. Ivacaftor was quantified by quadrupole time-of-flight mass spectrometry (Bruker Daltonik, Bremen, Germany) using D18 Ivacaftor as an internal standard (ivacaftor m/z= 391.2, D18-Ivacaftor m/z= 409.3). The injected volume was 4 µl. The detection limit was 2 µg/ml for lumacaftor and 0.2 µg/ml for ivacaftor.

The study protocol was approved by the ethics committees of all participating centers. Written informed consent was obtained from all patients included in the study, their parents or legal guardians.

Results

In total, 51 p.Phe508del homozygous CF patients took lumacaftor and ivacaftor within this post-approval study. Eight to sixteen weeks after the initiation of treatment with CFTR modulators blood was taken to determine the serum concentrations of lumacaftor and ivacaftor (Supplementary Table S1). The median serum levels were 0.5 µg/ml for ivacaftor and 24 µg/ml for lumacaftor (Table 1), comparable to data from the EMA assessment report [8]. The serum samples contained on average 50-fold more lumacaftor than ivacaftor. Figure 1 shows the ivacaftor and lumacaftor concentrations for the 49 samples with less than 2 µg/ml ivacaftor, the remaining two samples contained high levels of ivacaftor of 9 and close to 4 µg/ml, respectively (Supplementary Table S1). The data in Figure 1 demonstrate the lack of any correlation between ivacaftor and lumacaftor levels.

The serum levels of lumacaftor, ivacaftor and the lumacaftor/ivacaftor ratio were tested for association with anthropometry, spirometry and CFTR activity determined within 0.1 – 2.5 hours past blood sampling. In 14 of 15 tests no significant correlations between drug serum levels and clinical parametrs. Neither body mass index, FEV1, sweat chloride concentration, Sermet score [10] of the NPD and the chloride secretory response [9] in the ICM (Table 2) correlated. One exceptions from this general finding was noted, i.e. the serum level of ivacaftor correlated with the decrease of sweat chloride concentration ($P = 0.02$). The correlation of the ratio of lumacaftor to ivacaftor levels with the increase of the Sermet score towards the normal range in the NPD showed a trend ($P = 0.06$).

Discussion

Our multicenter study revealed that combination therapy of Phe508del homozygous individuals with CF with

lumacaftor/ivacaftor leads to highly variable serum concentrations of the two drugs that are not correlated with each other. Ivacaftor and lumacaftor have half-lives of 12 and 23 hours, respectively [8]. Since our study participants administered the morning dose according to protocol and blood was subsequently collected after a defined time lag with low inter-patient variability, different time points of blood sampling after dosage can hardly explain the large patient-to-patient variation of drug concentrations. We therefore speculate that the individual genetic repertoire of the polymorphic cytochrome P450 superfamily, drug-drug interactions and a variable body composition, particularly of the fat compartment, may give rise to a broad distribution of absorption, residence time, metabolism and excretion of these hydrophobic drugs.

According to our knowledge three groups have published protocols to determine the concentration of lumacaftor or ivacaftor by LC-MS in primary cells [6], sputum [4] and serum [4 -7] from in total 13 CF patients [4-7]. Our data on samples from 51 Phe508del homozygotes CF patients corroborate the previous reports that serum concentrations of ivacaftor during concomitant medication with lumacaftor are substantially lower than during ivacaftor monotherapy confirming the manufacturer's report [8] that lumacaftor is a CYP3A4 inducer that leads to a more rapid turnover of the CYP3A4 substrate ivacaftor.

With the exception of the correlation between ivacaftor levels and the change in sweat chloride, the absolute drug levels of lumacaftor or ivacaftor did not correlate with any improvement in anthropometry, lung function or CFTR activity in sweat gland, respiratory or intestinal epithelium. The remaining ivacaftor levels in presence of lumacaftor might therefore be of importance. However, our data indicate that the absolute blood levels of lumacaftor or ivacaftor seem to be no informative biomarkers to predict clinical improvement or the attenuation of the CF basic defect by combination therapy with these CFTR modulators. These findings are consistent with previous reports containing smaller numbers of ICM and NPD [12].

Nevertheless, drug monitoring of lumacaftor/ivacaftor may still be helpful to assess the patient's adherence to the prescribed treatment. Further studies are required to assess whether the CFTR correctors elexacaftor and tezacaftor [13-17] exert a similar pharmacokinetic behavior as observed for ivacaftor and lumacaftor.

Ethics approval

The study protocol was approved by the ethics committee of Hannover Medical School (No. 2846-2015) and subsequently by the ethics committees of the University of Heidelberg and the University of Giessen.

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Declaration of Competing Interest

C.D., R.T., A.M.D, S.Y.G, J.M.C., and C.D.E have nothing to disclose

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Supplementary Materials

Table 1. Serum levels of lumacaftor and ivacaftor in 51 p.Phe508del homozygous individuals with CF

	Median (IQR)	Range
Ivacaftor [µg/ml]	0.5 (0.3 - 0.8)	0.1 - 9.0
Lumacaftor [µg/ml]	23.5 (17.9 - 33.3)	0.0 - 52.2
Lumacaftor/Ivacaftor	50.8 (35.8 - 82.0)	0 – 390

Table 2. Correlation of ivacaftor and lumacaftor serum levels with the change to baseline in clinical features and CFTR activity*

	Δ BMI	Δ FEV1 predicted	Δ Sweat chloride	Δ Sermet score	Δ IBMX/ Forskolin
Ivacaftor	-0.012 (0.94)	0.008 (0.96)	0.334 (0.02)	-0.004 (0.98)	-0.059 (0.71)
Lumacaftor	0.143 (0.33)	0.077 (0.60)	0.169 (0.25)	0.066 (0.67)	0.127 (0.42)
Luma/Iva	0.003 (0.98)	0.166 (0.25)	-0.001 (0.92)	0.279 (0.06)	0.077 (0.62)

*For each entry the Table first denotes the correlation coefficient followed by the P value (Pearson correlation test; in brackets)

Figure 1. Serum levels of lumacaftor and ivacaftor of p.Phe508del homozygous individuals with CF during combination therapy