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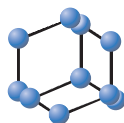
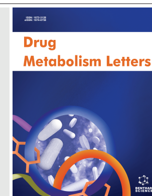
CASE REPORT





Cytochrome P450 3A4 Induction: Lumacaftor *versus* Ivacaftor Potentially Resulting in Significantly Reduced Plasma Concentration of Ivacaftor

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Abstract: ***Background & Objective***: Since the release of ivacaftor-lumacaftor, several red-flags have been raised that highlight the clinical efficacy of this combination strategy that may be limited due to antagonistic drug-drug interactions.

Method: The effect of ivacaftor, its major metabolites M1 and M6, lumacaftor and the novel cystic fibrosis transmembrane conductance regulator (CFTR) modulator tezacaftor at 10 µg/mL on the enzymatic activity of the major xenobiotic metabolizing enzymes CYP1A2 and CYP3A4 as well as the minor enzymes CYP2B6 and CYP2C9 was assayed.

Results: Lumacaftor ($3.74 \times 10^5 \pm 3.11 \times 10^4$ RLU), and ivacaftor-M6 ($3.43 \times 10^5 \pm 7.61 \times 10^3$ RLU) markedly induced the activity of CYP3A4. Ivacaftor ($2.22 \times 10^5 \pm 3.94 \times 10^4$ RLU) showed a lower relative ratio of luminescence units compared to chloramphenicol ($3.17 \times 10^5 \pm 1.55 \times 10^4$ RLU). Interestingly, ivacaftor-M1 ($6.74 \times 10^4 \pm 3.09 \times 10^4$ RLU) and the novel CFTR modulator tezacaftor ($2.40 \times 10^4 \pm 8.14 \times 10^4$ RLU) did not show CYP3A4 induction. In the CYP1A2 and CYP2C9 assay, all metabolites showed a decrease in the ratio of luminescence units compared to the controls. Ivacaftor, its major metabolites, lumacaftor and tezacaftor all showed a slight increase in the ratio of luminescence units compared to the control rifampin with CYP2B6.

Conclusion: All in all, present findings would suggest that lumacaftor and ivacaftor-M6 are strong inducers of CYP3A4, potentially reducing ivacaftor concentrations; ivacaftor itself induces CYP3A4 to some extent.

Keywords: Cystic fibrosis, ivacaftor, lumacaftor, CFTR modulator, cytochrome, drug interactions.

Drug Metabolism Letters

1. INTRODUCTION

Cystic Fibrosis (CF) is an autosomal recessive genetic life limiting disease that is caused by defective or deficient cystic fibrosis Transmembrane Conductance Regulator (CFTR) protein [1]. Ivacaftor (Kalydeco) is the first FDA-approved CFTR modulator drug, with evidenced clinical efficacy producing a significant improvement in the lung function over placebo in CF patients bearing the G551D-CFTR missense mutation which is found in 4-5% of the CF population [2, 3]. Unfortunately, ivacaftor is not effective in patients with the more common homozygous F508del mutation which results in misfolded CFTR, seen in ~28% of the CF population. Vertex gained approval for ivacaftor-lumacaftor (Orkambi), which combines ivacaftor with the CFTR corrector drug lumacaftor and has recently gained FDA approval of their new CFTR corrector tezacaftor. The clever strategy of combining a CFTR corrector which rescues F508del-CFTR to the cell surface with a modulator which potentiates CFTR channel activity, effectively expands the treatment window to the majority of the CF population [4]. Since the release of ivacaftor-lumacaftor combination, several red-flags have been raised that highlight the clinical efficacy of this clever combination strategy maybe be limited due to antagonistic drug-drug interactions (*i.e.* a case of lumacaftor *versus* ivacaftor) [5]. Firstly, unlike the experience with ivacaftor monotherapy, patients receiving ivacaftor-lumacaftor combination therapy displayed only modest improvements in lung function and pulmonary exacerbations [6]. Secondly, evidence has emerged from a number of independent laboratories that suggests that prolonged exposure to ivacaftor counteracts the corrector function of lumacaftor, by destabilizing the lumacaftor rescued mature glycoform of F508del-CFTR and reducing its surface expression [7, 8]. Such inhibitory interactions are especially

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concerning given that lumacaftor only partially restores (11-15%) F508del CFTR surface expression [7, 8]. Thirdly, ivacaftor and lumacaftor are both very hydrophobic drugs and as such are ~99% bound to plasma proteins; which significantly limits the free (active) drug concentration [9, 10]. Lastly, clinical reports hint that lumacaftor may be an inducer of cytochrome (CYP) P450 drug metabolizing enzymes and paradoxically ivacaftor is a substrate of CYP3A4 [11-13]. Moreover, the product information sheet for ivacaftor-lumacaftor strongly contraindicates co-administration of strong CYP3A4 inducers such as rifampin or St. John's Wort [11, 12]. Together, this would suggest potential antagonistic drug-drug interactions between lumacaftor and ivacaftor is at play where the former induces the metabolism of the latter. Overall, these factors maybe compounding together to limit the clinical efficacy of ivacaftor-lumacaftor therapy.

To investigate the impact of inter-individual variability of the ivacaftor monotherapy of ivacaftor-lumacaftor treatment due to cytochrome P4503A4 metabolism [6, 11, 14, 15], we have measured the plasma concentrations of ivacaftor and its active metabolite M1 (hydroxymethylivacaftor) and M6 (ivacaftorcarboxylate) and lumacaftor in human plasma in two compliant female patients: patient one treated with 150mg/q12h ivacaftor and patient two treated with 200mg/q12h lumacaftor/125mg/q12h ivacaftor (patient samples were previously used to validate the LC-MS assay to FDA standards [16]. Both patients did not receive any CYP3A4 inducers. Plasma samples were collected at steady state after >2 weeks of treatment and 2.5 h after dosing. Sputum samples were ~2-3 mL and collected over 2-3 h, post-dosing. Drug concentrations in patient plasma and sputum samples were determined by LC-MS (Table **1**) [16, 17].

Our findings confirm that there was no impact of combination therapy on the M6 concentration in patient 2 (0.15 µg/mL) which is comparable to the concentration in patient 1 (0.16 µg/mL) who received ivacaftor monotherapy. However, a major decrease in ivacaftor plasma concentrations was detected: 0.97 µg/mL in patient 1 compared to the levels 0.06 µg/mL in patient 2. Similarly, a decreased level of the active M1 metabolite was detected in patient 2 0.07 µg/mL, compared to patient 1 0.50 µg/mL. Surprisingly high lumacaftor plasma concentrations of 3.69 µg/mL were detected in patient 2. It seems contradictory that the ivacaftor concentration has been reduced from 150mg/q12h *per se* to 125mg/q12h when given with the CYP inducer lumacaftor. As lumacaftor improves the trafficking of the CFTR and not the gating activity, lumacaftor alone does not provide enough improvement for clinical benefit, the question presents itself whether the plasma concentration of ivacaftor in the ivacaftor-lumacaftor patient is clinically efficious (Table **1**)?

The effect of ivacaftor, its major metabolites M1 and M6, lumacaftor and the novel CFTR modulator tezacaftor at 10 µg/mL on the enzymatic activity of the major xenobiotic metabolizing enzymes CYP1A2 and CYP3A4 as well as the minor enzymes CYP2B6 and CYP2C9 was assayed (Fig. **1**). Measurement of the cytochrome activity *in vitro* was determined using the P450-Glo luminescence assay (Promega) according to the manufacturer's instructions. Human recombinant enzymes were purchased from Sigma-Aldrich. In brief, for the P450-Glo assays, a 50 µL reaction mixture containing CYP enzyme 400 mM KPO_4 buffer and a luminogenic CYP-specific substrate, were mixed with an equal volume of test compound and the mixture was incubated at room temperature for 10 min. Following incubation, an equal volume of NADPH regenerating system solution (2.6 mM NADP+, 6.6 mM glucose-6-phosphate, 6.6 mM $MgCl_2$ and 0.8 U/mL glucose-6-phosphate dehydrogenase) was added. The reaction mixture was incubated at room temperature for 30 min. Luciferin detection reagent was then added and the mixture was incubated at room temperature for 20 min. The luminescence was then measured using a micro plate luminescence PerkinElmer EnSight Plate Reader. Lumacaftor

Table 1. Drug and metabolite concentrations in plasma and sputum in CF patients receiving Kalydeco and Orkambi therapy.

Medication Management					
Patient 1	150mg/q12h[#] Ivacaftor, 750 mg ciprofloxacin bd[Δ], inhaled tobramycin 112mg/bd 250/25µg bd seretide, mometasone nasal spray 2 sprays/bd, 100 µg salbutamol 2 puffs bd plus prn[§], pancreatic extract tds[ǂ] with meals, 500mg/d Calcium/400IU/d Vitamin D bd, 600mg salt prn				
Patient 2	200mg/q12h Lumacaftor and 125mg/q12h Ivacaftor Pancreatic extract, 25µg/d vitamin D, 20mg/d pantoprazole, 300mg/d nizatidine, 500mg/m/w/f azithromycin, hypertonic saline 6%, 5mL bd, 250/25 mg/d salmeterol, 2x/d Vitamin ABDECK, 2 salt tablets, 2mg/35mcg /d ethinyloestradiol/cyproterone acetate, 100mcg/prn salbutamol, inhaled tobramycin				
Plasma concentrations [16] **					
		IVA [µg/mL]	IVA M1 [µg/mL]	IVA M6 [µg/mL]	LUMA [µg/mL]
KALYDECO Patient 1	Plasma c_{ss}	0.73±0.02	0.41±0.08	0.11±0.03	--
	Plasma 2.5h	0.97±0.31	0.50±0.09	0.16±0.04	--
ORKAMBI Patient 2	Plasma 2.5 h	0.06±0.01	0.07±0.01	0.15±0.03	4.42±0.67

[#]Every 12 hours; [Δ] twice daily; [§] as needed; ǂ 3 times per day; *below the limit of quantification (LOQ)
**Plasma concentrations were first reported by Schneider *et al* (2016) [16].

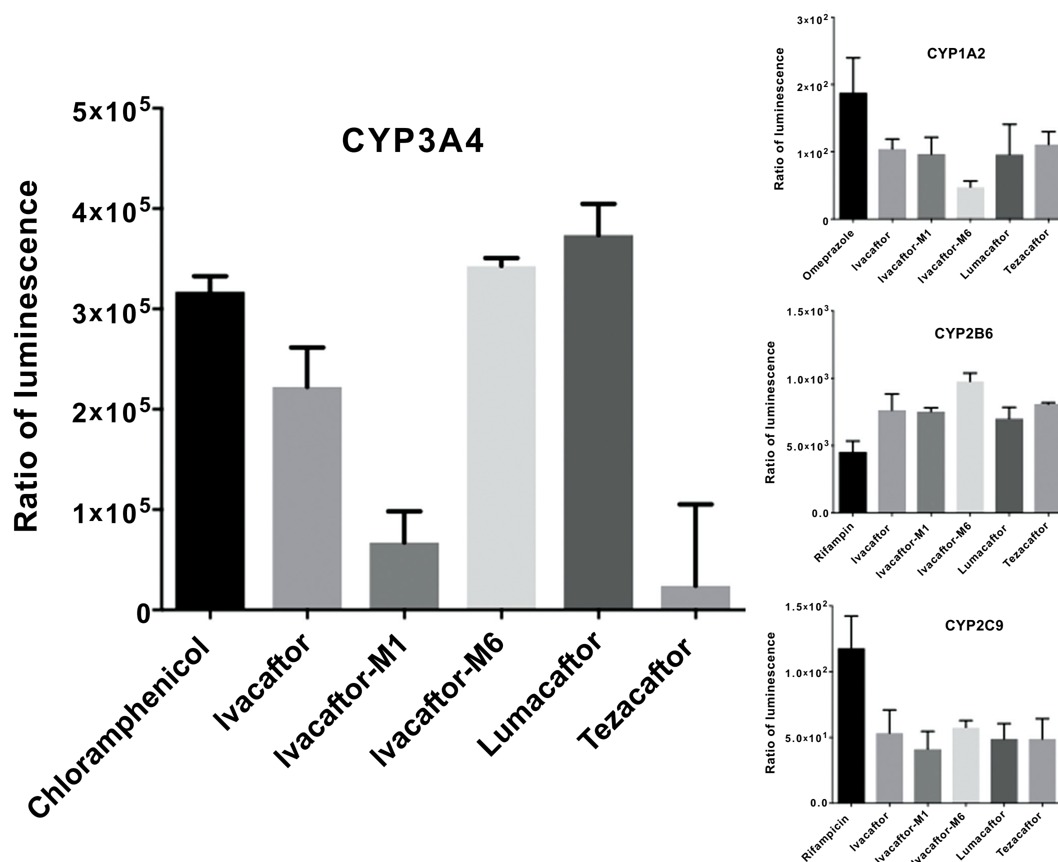

Fig. (1). Effect of ivacaftor, ivacaftor-M1, ivacaftor-M6, lumacaftor and tezacaftor on the activity of CYP3A4 (right hand side: CYP1A2, CYP2B6 and CYP2C9) in a cell-free luminescence assay. The P450-Glo assay was performed using chloramphenicol (CYP3A4), omeprazole (CYP1A2), rifampin (CYP2B6) and rifampicin (CYP2C9) at 10 µM as a positive control (cytochrome induction). Data is the mean of three independent measurements. Luminescence ratio units are corrected for the enzyme + vehicle background. Data are presented as relative luminescence units and are expressed as the mean ± SD (n=3).

($3.74 \times 10^5 \pm 3.11 \times 10^4$ RLU), and ivacaftor-M6 ($3.43 \times 10^5 \pm 7.61 \times 10^3$ RLU) markedly induced the activity of CYP3A4 (Fig. **1**). Ivacaftor ($2.22 \times 10^5 \pm 3.94 \times 10^4$ RLU) showed a lower relative ratio of luminescence units compared to chloramphenicol ($3.17 \times 10^5 \pm 1.55 \times 10^4$ RLU). Interestingly, ivacaftor-M1 ($6.74 \times 10^4 \pm 3.09 \times 10^4$ RLU) and the novel CFTR modulator tezacaftor ($2.40 \times 10^4 \pm 8.14 \times 10^4$ RLU) did not show CYP3A4 induction. In the CYP1A2 and CYP2C9 assay, all metabolites showed a decrease in the ratio of luminescence units compared to the controls omeprazole ($1.88 \times 10^2 \pm 5.19 \times 10^1$ RLU), and rifampicin ($1.18 \times 10^2 \pm 2.46 \times 10^1$ RLU), respectively. Ivacaftor, its major metabolites, lumacaftor and tezacaftor all showed slight increase in the ratio of luminescence units compared to the control rifampin ($4.52 \times 10^2 \pm 8.06 \times 10^1$ RLU) with CYP2B6.

We have previously reported that co-medication with other CF drugs could impact the free plasma levels of ivacaftor, either *via* displacement from plasma binding proteins or induction or inhibition of cytochrome P450 metabolism [10]. Ciprofloxacin and other quinolone antibiotics have been reported to as potent CYPIA2 inhibitors [18]. Some evidence was reported that ciprofloxacin inhibits CYP3A4, albeit at the extraordinarily high concentration of 2 mM, which is not remotely achievable in patients (C_{max}= 2.34 ±1.15 µg/mL) [19, 20]. Although, patient 1 was taking ciprofloxacin (750 mg/bd), given the very weak inhibitory activity of ciprofloxacin against CYP34A, this co-medication is unlikely to be the cause of the higher ivacaftor plasma concentrations observed in this patient's plasma.

All in all, present findings would suggest that lumacaftor and ivacaftor-M6 are strong inducers of CYP3A4, potentially reducing ivacaftor concentrations; ivacaftor itself induces CYP3A4 to some extent. Promisingly, the new CFTR modulator tezacaftor and also ivacaftor-M1 did not show CYP3A4 induction. Ivacaftor, its major metabolites, lumacaftor and tezacaftor all showed slight induction of CYP2B6 and no induction of CYP1A2 or CYP2C9. As lumacaftor therapy alone did not result in significant patient outcomes, the question remains if the concentrations of ivacaftor in ivacaftor-lumacaftor therapy are above the therapeutic threshold and if therapeutic drug monitoring protocols should be put in place [21]. This strategy would lead to better patient outcomes and greatly benefit patients.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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Declared none.

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