Development and use of biomarkers in clinical development of new therapies for chronic airway disease

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CHAPTER 7

REPEATED DOSING OF RPL554, A NOVEL INHALED PHOSPHODIESTERASE 3 /4 INHIBITOR, ELICITS SUSTAINED BRONCHODILATOR EFFECTS IN ALLERGIC ASTHMATICS

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Efficacy and safety of RPL554, a dual PDE3 and PDE4 inhibitor, in healthy volunteers and in patients with asthma or chronic obstructive pulmonary disease: findings from four clinical trials

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ABSTRACT

AIM To investigate the safety, tolerability and effect of repeated daily doses of nebulized RPL554, a dual phosphodiesterase 3/4 inhibitor.

METHOD Thirteen clinically stable allergic asthmatics received placebo on the first day and subsequently nebulized RPL554 (0.018 MG/KG) for 6 consecutive days in a single blind manner. FEV1 was measured during 6 hours after administration.

RESULT The mean estimated half-life of RPL554 in plasma was 7.4 hours. The median (range) value for accumulation on Day 6, based on AUCT ratio, was 1.17 (0.64 to 2.28). Repeated dosing of nebulized RPL554 caused consistent bronchodilation on all 6 consecutive days. An increase in mean FEV1 after RPL554 compared to placebo was 312.9, 300.5 and 275.4 ML on day 1, 3 and 6 respectively. The maximum increase in FEV1 compared to placebo on day 1, 3 and 6 was 555, 505 and 485 ML, respectively. Only a small decline in FEV1 of 38 ML (95%CI:-95.9; 20.9 ML) or 12% on day 6 compared to day 1 and corrected for placebo was observed. The most frequent reported adverse events were headache, irritation of the larynx and dryness of the throat. Heart rate increased with 5 BPM (95%CI: 3.6; 7.4) and diastolic blood pressure decreased with 3.3 MMHG (95%CI: -5.6; -1.1) on day 6 compared to placebo.

CONCLUSION Repeated daily doses of nebulized RPL554 0.018 MG/KG in allergic asthmatics for 6 days showed consistent bronchodilator effects and was generally well tolerated.

INTRODUCTION

Treatment of asthma currently consists of two distinct categories of drugs: relievers like β2 agonists which are used on an as needed basis and act quickly to reverse bronchoconstriction and relieve symptoms, and controllers like glucocorticosteriods which are taken daily on a long term basis to keep the asthma under clinical control [1]. A potentially new group of anti-asthmatics drugs is the selective phosphodiesterase (PDE) 3/4 inhibitor which may have both bronchodilation effects and anti-inflammatory properties. PDE3 inhibition produces bronchodilation [2;3], while PDE4 inhibition results in anti-inflammatory and disease modifying features as PDE4 regulates the function of several immune, inflammatory (neutrophils, eosinophils) and structural cells (E.G. airway smooth muscle) involved in the pathophysiology of chronic inflammatory diseases like asthma [4]. It has been suggested that PDE3 and PDE4 together act synergetic as each pathway affects the function of different cell types [5]. The dual PDE3/4 inhibitors studied so far in humans are all associated with unfavorable side effects [6;7], such as central nervous system (CNS) and gastrointestinal complaints.

To prevent these systemic side effects, RPL554, a novel dual PDE3/4 inhibitor to be administered by inhalation, was developed for the treatment of asthma. As RPL554 is administered directly into the airways, systemic exposure will to be low. RPL554 is a trequinsin analogue with human IC50 values for PDE3 and PDE4 of 400 PM and 1.5 μM respectively [8]. It has shown bronchodilation, bronchoprotective and anti-inflammatory properties in animal models of allergen-induced asthma and rhinitis. Safety and bronchodilation characteristics were investigated for the first time in humans at the Centre for Human Drug Research in Leiden, the Netherlands; RPL554 significantly produced bronchodilation at doses between 0.009 and 0.072 MG/KG with a mean maximal increase of 520 ML (95%CI: 320; 720 ML) in patients with mild to moderate asthma without CNS and gastrointestinal side effects. During methacholine challenge tests, RPL554 led to an increase of approximately 1.46 doubling doses change from baseline (95%CI: 0.63; 2.28) compared to placebo demonstrating both bronchoprotective and bronchodilative properties of RPL554 in asthmatic patients. The combination of both bronchoprotective and bronchodilatory properties with the absence of CNS and gastrointestinal side effects is promising for the further development of RPL554 for the treatment of allergic asthma.

Asthma therapy often starts at a young age and is given over many years. As continuous use of medication can be associated with a decline in drug effectiveness, longer term effectiveness of RPL554 might be a concern. Therefore, the next step in the development of RPL554 as a potential novel treatment for allergic asthma is to investigate RPL554's effectiveness after repeated administrations. In the present study, lung function is assessed after repeated daily dosing of RPL554 for 6 consecutive days, to investigate the sustainability of effect on FEV1.

METHODS

DESIGN The study consisted of a single arm active treatment, assessing the bronchodilator effects of 6 repeated inhaled daily doses of RPL554 (0.018 MG/KG) in 13 patients with allergic asthma in a single blind manner. Visits to the clinic occurred at screening and at study days: Day -1 for baseline assessments and placebo administration, Day 1 to 6 for daily dosing of RPL554, and Day 7 for follow-up.

Spirometry with frequent FEV1 measurements was performed on all study days, *Figure 1*. Subjects only proceeded to the next dosing day, when stable and no clinically relevant adverse events had occurred. The study protocol was approved by the medical ethical committee Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO) in Assen, the Netherlands and conducted according to the principles of the "Declaration of Helsinki" and the pertaining Dutch law.

SUBJECTS Thirteen male subjects with mild to moderate persistent clinically stable allergic asthma participated in the study. All subjects were non-smoking or ex-smokers (> 6 months abstinence and < 10 pack years) and did not suffer from any other significant disease. Patients only used short acting β2-agonists pro re nata, had a FEV1 of > 70% of predicted, a positive provocation test to inhaled Methacholine with a fall in FEV1 of > 20% (PC20MCH) after < 8 MG/ML, methacholine, and a positive skin prick test (SPT > 3MM) for at least one allergen (grass or tree pollen, house dust mite, D. Farinae, cat, dog, or horse-dander, Aspergillus Fumigatus, A. Alternata or Artemisia Vulgaris). FEV1 values at screening and day -1 were within 10% of each other. Patients had a documented reversibility in lung function of > 12% or 0.2 L increase in FEV1 after 200 μg salbutamol and had no history of respiratory tract infections at least 3 weeks before enrollment and during the study. They had not, except for occasional inhaled short acting β2-agonists which was not allowed within 8 hours before start of the study days, used concomitant anti-asthma or anti-allergy medication in the weeks prior to the screening.

TREATMENT RPL554 is 9,10-Dimethoxy-2-(2,4,6-trimethylphenylimino)-3-(Ncarbamoyl-2-aminoethyl)-3,4,6,7-tetrahydro-2H-pyrimido[6,1-a]isoquinolin-4-one powder and was diluted in a citrate-phosphate buffer of pH 3.2. The same buffer was used for placebo. Drug administration took place by oral inhalation of nebulized RPL554 (0.018 MG/KG and based on an average weight of 65-70 KG) through a mouth piece connected to a calibrated electronic nebuliser (Clement-Clarke International AC4000, UK) while the nose was clipped. The drug solution was nebulized from a 0.6 MG/ML solution at a rate of 0.2 ML/minute during 10 minutes.

SAFETY Safety evaluation contained the recordings of adverse events, physical examinations, blood chemistry, haematology and coagulation (analysed at the Leiden University Medical Center), twelve lead ECG, blood pressure and heart rate measurements. Blood pressure and heart rate measurements were conducted daily by automated oscillometric equipment (BMK-1101K; Nihon Kohden, Japan, or Dash 4000; GE Healthcare, USA). Daily Twelve-lead ECG recordings were made using Electrocardiograph Marquette 5000/5500 (USA).

PHARMACOKINETICS Plasma samples, to assess the concentration of RPL554, were drawn at regular intervals (t = 0, 0.25, 0.5, 1, 2 and 4 hours post dose on days 1 up and till 6 and in addition at 6 hours on days 1, 3 and 6) in 4 ML EDTA tubes. The samples were centrifuged within one hour for 10 minutes at 4° C, 2000G, and stored until analysis at -70 °C. RPL554 plasma concentrations were determined with a validated HPLC method by ABL bv, Assen, The Netherlands.

PHARMACODYNAMICS PULMONARY FUNCTION TESTS Lung function tests were performed with the Vmax 20C pulmonary spirometer (SensorMedics, USA) at screening. A portable spirometer ZAN 100 (Accuramed, Belgium) was used during study days. At approximately 15 and 5 minutes predose, baseline FEV1 measurements were performed and calculated as the average of the 2 highest out of 3 FEV1 measurements (within 5%). Post-dosing measurements were performed in duplicate at regular intervals (t = 0, 0.25, 0.5, 1, 1.5 and 2 hours post dose on study days 1 up and till 6, and in addition on 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 hours on study days 1, 3 and 6) of which the highest, technically satisfactory FEV1 was included into analysis.

METHACHOLINE BROMIDE CHALLENGES (MCH) Methacholine challenge performed at screening was used to determine the degree of non-specific bronchial reactivity. MCH challenges were performed by tidal breathing method according to a standardized protocol [9;10]. In short, three technically satisfactory FEV1 measurements within 5% were performed pre and post diluent; the mean of the 2 highest FEV1 values was included in analyses. The post diluent baseline had to remain within 10% of the pre diluent baseline, or otherwise the subject would be excluded from the study. Methacholine bromide (MBR) (Janssen Pharmaceutica, Belgium) was aerosolized by a DeVilbiss 646 jet nebuliser (output 0.13 ML/minutes, Devilbiss Healthcare, inc, Somerset, USA) and inhaled during 2 minutes tidal breathing with the nose clipped. Consecutive MBR doses (0.0625-16 MG/ML) were inhaled at 5 minutes intervals and airway response was measured in duplicate following each dose (and expressed as percentage decline from post-diluent baseline FEV1). The procedure was discontinued after a fall in FEV1 of at least 20% from baseline occurred or the highest permissible dose had been inhaled.

PHARMACOKINETIC ANALYSIS The pharmacokinetic analyses were based on a non-compartmental analysis and calculated in Phoenix WinNonLin 6.2. CMAX and TMAX were determined from visual inspection of the plasma concentration-time profile. The area under the plasma concentration-time curve from zero to the time of the last quantifiable plasma concentration, AUC0-t, was calculated using the linear and log trapezoidal rule. AUCT was defined as the AUC from zero

to 24 hours post dose on each dosing day. The rate constant of the slowest disposition phase (λz) was calculated by log-linear regression of the terminal portion of the concentration-time profile. The terminal half-life (t½) was derived from the equation ln(2)/ λz. Total apparent drug clearance (CL/F) and volume of distribution (Vz/F) were estimated by dividing the dose by AUCt, and product of λz and AUC, respectively. The observed accumulation ratio (Rac) was estimated as follows: I) from the observed ratio (AUCt/ AUC0-24) where τ was 48 to 72 h (Day 3) and 120 to 144 h (Day 6), and II) from the observed ratio (Cmin, day n/Cmin,d2) where n = Day 3 and Day 6 respectively. The used Cmin reflects the pre-dose plasma concentration measured prior to dosing on the given day.

PHARMACODYNAMIC ANALYSIS To compare the FEV1 measures for 6 hours post dosing at day 1, day 3 and day 6, FEV1 results normalized for each individual to FEV1 at day -1 were analysed using a mixed model repeated measures analysis of variance with day, time, day by time as fixed factors and subject, subject by time and subject by day as random factors. The difference between two days (-1 and 1, -1 and 3, -1 and 6, 1 and 3, 1 and 6, 3 and 6) were used as contrasts and expressed as estimated least square mean difference in RPL554 effect, together with the corresponding estimated percentage difference, the 95% confidence interval and the p-value.

POWER CALCULATION A priori, the sample size of the study, as calculated with nQuery Advisor V5.0 (Statistical Solutions, Ltd, Cork, Ireland), was based upon intra individual variability in FEV1 obtained from previous studies with single doses of RPL554. Using a standard deviation of the differences of 180 mL, a decrease of 50% in treatment effect between day 1 and day 6 (corresponding to around 150 mL decrease in the drug response over 6 hours) could have been detected with a power of 80% and a significance level of 5% and 14 subjects. After 8 subjects the SD of the differences between days was estimated. This estimate revealed an SD of 97 mL leading to a subsequent recalculated sample size of 12 patients which would be an adequate number to detect a difference in treatment effect of 87 mL.

RESULTS

DEMOGRAPHICS A total of 13 patients with mild to moderate persistent clinically stable allergic asthma were recruited and received RPL554 of which 12 subjects completed the study, *Table 1*. One subject withdrew his consent on day 1. His results were included into safety and pharmacokinetic analysis but were excluded from the pharmacodynamic analysis. The reversibility test, using inhalation of 200 µg salbutamol, showed an average increase in FEV1 of 0.49 L or 12.7%.

SAFETY AND TOLERABILITY The most common adverse event were headache (n= 4), dizziness (n = 3) and larynx irritations (n=3). All events occurred as single events, were mild in intensity and transient. *Table 2* provides an overview of the most important vital signs and ECG parameters. Heart rate increased around 5 beats per minute and the diastolic blood pressure decreased with 3.3 mmHg on day 6 compared to placebo (day -1). Furthermore, a decrease in the QT interval compared to placebo emerges, which disappears after applying Frederica's correction method. In addition, there were no serious adverse events and no relevant changes observed in laboratory safety assessment and on the ECG parameters: RR-, PR-, QRS- and QT-intervals.

PHARMACOKINETICS Results of RPL554 plasma concentrations are depicted in *Figure 2* and the corresponding pharmacokinetic parameters are shown in *Table 3*.

PHARMACODYNAMIC Repeated dosing of nebulized RPL554 caused bronchodilation on all 6 consecutive days, *Figure 3* and *Figure 4*. The increase in mean FEV1 after RPL554 compared to placebo was 312.9, 300.5 and 275.4 mL on day 1, 3 and 6 respectively. The maximum increase in FEV1 compared to placebo on day 1, 3 and 6 was 555, 505 and 485 mL, respectively. The differences in the least square mean for the change in FEV1 corrected for placebo, between day 3 and day 1 was -4% or -12.4 mL, (p = 0.66, 95%CI:-70.9; 46.0 mL) and between day 6 and day 1 was -12 % or -37.5 mL (p = 0.20, 95%CI:-95.9; 20.9 mL). The corresponding estimated difference for the maximum FEV1, corrected for placebo, between day 3 and day 1 was -9.0% or -50.0 mL (p = 0.11, 95%CI: -113; 12.9 mL) and between day 6 and day 1 was -12.6% or -70 mL (p = 0.03; 95%CI:-133; -7.1 mL). On day 6, FEV1 results were characterised by a small decline in lung function at around 3 hours after dosing which was apparent in 10 of the 12 evaluated patients on day 6 and absent on other days of the study.

DISCUSSION

Previous studies with single doses of nebulized RPL554 showed strong bronchodilation and possible bronchoprotective effects in asthmatic patients, without significant side effects. These data are promising for the further development of RPL554 as a potential treatment of allergic asthma.

As asthma is a chronic condition, continuous drug use is often required. Tolerance for asthma medication has been described before for short-acting β2 adrenoceptor agonists, however it has generally been considered as non-clinically significant [11]. For the further development of RPL554 as a potential anti-asthma medication which inhibits PDE3/4, we investigated whether RPL554 did not lose its effectiveness, and studied especially the occurrence of acute tolerance, after repeated daily dosing. We report that six repeated daily doses of nebulized RPL554 (0.018 MG/KG) in allergic asthmatics was well tolerated and resulted in sustained bronchodilation.

Bronchodilation effects, expressed as mean and maximum increase in FEV1 compared to placebo, were sustained throughout the study. Nevertheless, a small decrease in FEV1 on day 3 and 6 and compared to day 1 was detected during the study period but felt within the observed standard deviation of the differences. The decrease in maximum FEV1 on day 6 compared to day 1 however was significant. We believe that during the time period of the study the decline in FEV1 did not influence the beneficial effects of RPL554 and was certainly too small to underpin the presence of acute tolerance, as the maximum increase in FEV1 of 485 ML on day six compared to placebo still matches up to the observed effects of salbutamol during the reversibility test.

It was observed that the FEV1 results on day 6 were characterised by a decline in lung function at around 3 hours after dosing. This decline was apparent in 10 of the 12 evaluated patients on day 6 and absent on other days of the study. However the observation was classified as spurious as no satisfactory explanation could be found; all procedures were identical during all study days, subjects had limited room to manoeuvre on a smoking free ward and lung function tests were performed by a team of experienced lung function technicians who worked in shifts in a random schedule throughout the study.

There was a minimal though variable accumulation of RPL554 which is reflected in the median ratio and range of RAC estimated using both AUCT and CMIN. The inter-patient variability noted in RAC was greater following estimation by CMIN ratios compared with the AUCT ratios. This observation likely reflects the variability inherent in a point estimate such as CMIN compared with the AUC parameter which represents PK behaviour over a period of time. The systemic exposure to RPL554, as reflected in low concentrations for CMAX and AUCT , can be regarded as little, which might explain the relative mild side effect profile. Indeed, only a few adverse events like mild headache and dizziness were reported. The observed decrease in diastolic blood pressure, the increase in heart rate and the subsequent decrease in QT-interval along the course of the study could be considered as a PDE-inhibitor-class-specific adverse event. The observed increase in heart rate possibly reflects a sympathetically mediated homeostatic reflex response to the observed tendency for a fall in blood pressure that occurs because of relaxation of vascular smooth muscle, a well-known effect of PDE3 inhibition (SMPC amrinone, milrone, olprinone and cilostazol). Furthermore, although the QT interval decreased as a result of heart rate increase, the QT interval as corrected with Fridericia's method remained stable, suggesting that RPL554 did not affect cardiac repolarization.

Although the design of the study provided the fundament to answer the primary objective on acute tolerance, the robustness of bronchodilation over longer period of time should be investigate in more extensive follow up studies, preferably with an active comparator. Given that duration of bronchodilation of a single dose of RPL554 lasts for several hours, a short acting β agonist would have been a reasonable contrast. However, the use of placebo on day -1 provided sufficient information to judge RPL554 on its own merits. The present study did not make use of a methacholine challenge as a measurement for tolerances, as the main outcome of this study was related to bronchodilation and not to bronchoprotection. However, as decreased protection against bronchoconstrictor stimuli like methacholine or histamine is a well-known effect of long term treatment with long acting β2 agonists [12;13], measuring bronchoprotection is something to consider in future studies with RPL554.

In conclusion, repeated daily doses of nebulised RPL554 0.018 MG/KG in allergic asthmatics for 6 days showed sustained bronchodilator effects during the study period and was generally well tolerated. The data suggest that acute tolerance to the effect of RPL554 does not develop during 6 days of continuous drug administration. Whether longer term treatment with RPL554 results in a clinically significant decreased effect, needs to be determined in subsequent clinical trials.

Table 1 Baseline Patient characteristics

Number of subjects	13
Age (years)	26 (19-52)
BMI (kg/m2)	23.0 (1.81)
FEV1 (l)	3.99 (0.66)
FEV1 PRED. (%)	88 (8.5)
FEV1/FVC (%)	73 (8.2)
PC20MCH (mg/ml)	1.68 (1.7)

All numbers are expressed in means (SD) expect age is expressed as mean (range)

Table 2 Vital signs and ECG parameters

	LMSS				Contrasts					
Parameter	Day -1	Day 1	Day 3	Day 6	Day 1 vs. Day -1	Day 3 vs. Day -1	Day -6 vs. Day -1	Day 3 vs. Day 1	Day 6 vs. Day 1	Day 6 vs. Day 3
Diastolic blood pressure (mmHg)	68.6	66.4	66.1	65.2	-2.1 (-4.4; 0.2) p=0.0670	-2.4 (-4.7; -0.2) p=0.0372	-3.3 (-5.6; -1.1) p=0.0052	-0.3 (-2.5; 1.9) p=0.7712	-1.2 (-3.4; 1.0) p=0.2572	-0.9 (-3.1; 1.3) p=0.3944
Systolic blood pressure (mmHg)	119.2	119.2	119.3	118.2	0.0 (-2.9; 2.9) p=0.9958	0.1 (-2.8; 3.0) p=0.9367	-1.0 (-3.9; 1.9) p=0.4962	0.1 (-2.7; 3.0) p=0.9391	-1.0 (-3.8; 1.9) p=0.4808	-1.1 (-3.9; 1.8) p=0.4355
Heart Rate: 12-lead ECG (bpm)	60.2	62.5	62.8	65.7	2.4 (0.4; 4.3) p=0.0180	2.6 (0.7; 4.5) p=0.0093	5.5 (3.6; 7.4) p=<.0001	0.3 (-1.0; 1.5) p=0.6807	3.2 (1.9; 4.4) p=<.0001	2.9 (1.6; 4.2) p=<.0001
QT interval (msec)	396.5	390.5	388.1	385.8	-6.1 (-11.8; -0.4) p=0.0379	-8.5 (-14.2; -2.8) p=0.0048	-10.7 (-16.4; -5.0) p=0.0005	-2.4 (-7.8; 3.0) p=0.3670	-4.7 (-10.1; 0.7) p=0.0870	-2.3 (-7.7; 3.2) p=0.3936
QTc Fridericia (msec)	394.8	393.5	392.0	395.7	-1.3 (-5.7; 3.1) p=0.5572	-2.8 (-7.2; 1.6) p=0.2072	0.9 (-3.5; 5.3) p=0.6770	-1.5 (-5.9; 3.0) p=0.4936	2.2 (-2.3; 6.6) p=0.3202	3.7 (-0.8; 8.1) p=0.1007

Contrast given as difference (95%CI) and p – value. LSM: least mean square

Table 3 Pharmacokinetic parameters of RPL554

	Day 1	Day 3	Day 6
	n = 13	n = 12	n = 12
CMAX (pg/ml)	2136 (1454)	2041 (1215)	2269 (1369)
CMIN (pg/ml)	NA	12 (7)	15 (12)
$T^1/_2$ (h)	NC	NC	7.4 (3.0)
AUCT (pg/ml)*	2111 (44%)	2056 (56%)	2730 (36%)
VZ/F* (L)	NC	NC	5117 (68%)
CL/F* (L/h)	NC	NC	498 (41) %
RAC using Cmin**	NA	1.26 (0.24-2.96)	1.18 (0.16-6.42)
RAC using AUC**	NA	0.90 (0.44-2.48)	1.27 (0.64-2.28)

Values given as mean (SD); *Values given as geometric mean (CV%) ; ** Values given as median (range);
AUCT : refers to post dose values, covering 0-24, 48-72 and 120-144 hours for day 1, 3 and 6 respectively.;
CMIN : refers to the pre dose concentration; VZ: apparent volume of distribution; CL: clearance; F: biological availability; RAC: accumulation ratio; NC: not calculated

Figure 1 Overview of study design. MCh: methacholine challenge. FEV1 was measured at frequent intervals during the study days

	SCREENING	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	FOLLOW UP
MCH	X								
REVERSIBILITY TEST	X								
FEV1	X	X	X	X	X	X	X	X	X
STUDY MEDICATION		PLACEBO	RPL554	RPL554	RPL554	RPL554	RPL554	RPL554	

Figure 2 RPL554 plasma concentrations with SD error bars

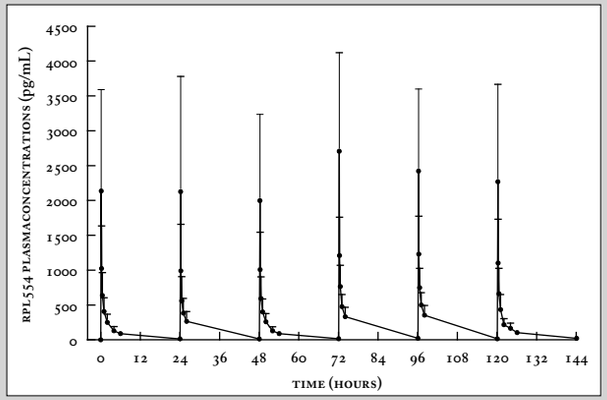


Figure 3 FEV1 time profile with SD error bars

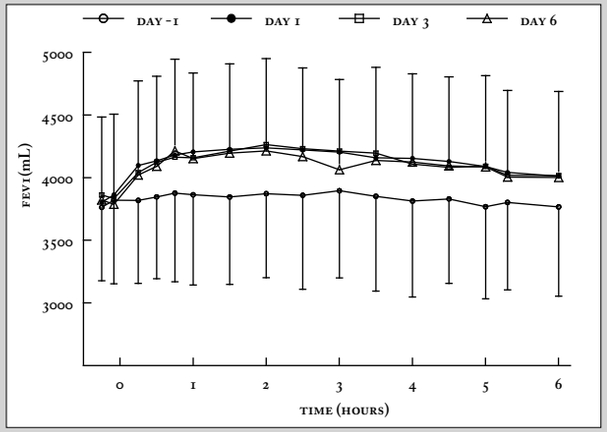
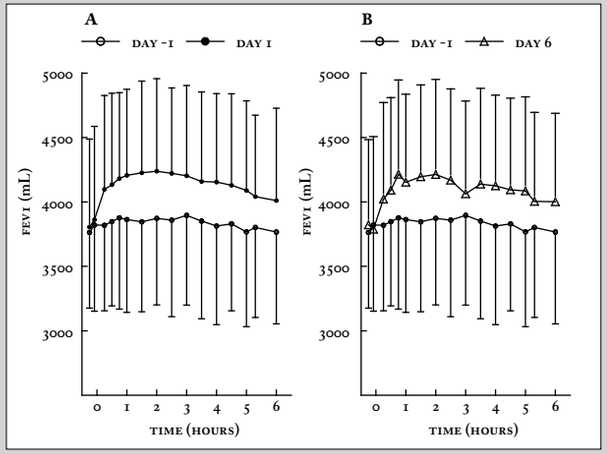


Figure 4A&B FEV1 time profile (with SD error bars) for placebo at day -1 and day 1 and for placebo at day -1 and day 6



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