Indirect bronchial provocation tests in childhood asthma
Kersten, Elin

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 7

Can a Single Dose Response Predict the Clinical Effectiveness of Montelukast in Adolescents with Asthma?

Elin T.G. Kersten
Anne M. Akkerman-Nijland
Jean M.M. Driessen
Zuzana Diamant
Bernard J. Thio

Submitted
ABSTRACT

Rationale
Exercise induced bronchoconstriction (EIB) can be prevented by a single dose of montelukast (MLK). This effect is variable, similar to the variable responsiveness observed after daily treatment with MLK. We hypothesized that the effect of a single MLK-dose (5 or 10 mg) on EIB could predict the clinical effectiveness of longer term once daily treatment.

Methods
This was a prospective, open-label study. Twenty-four asthmatic adolescents (12-17 y) suboptimally controlled by low dose inhaled corticosteroids, with ≥10% post-exercise fall in FEV₁, were included. They performed an exercise test at baseline, 20h after a single MLK-dose and 40-44h after the last dose of 4 weeks once daily treatment. The correlations between the effect of a single dose and 4 weeks treatment on area under the curve (AUC) and maximum % fall in FEV₁ were calculated.

Results
AUC_{0-20} decreased significantly after a single MLK-dose (P = 0.001, CI 64.9 – 218.2), but not after 4 weeks of treatment (P = 0.080, CI -12.2 – 200.4). There was a moderate correlation between the effect of a single MLK-dose and 4 weeks treatment on AUC_{0-20}, r = 0.49 (P = 0.011), and maximum % fall in FEV₁, r = 0.40 (P = 0.035). The positive and negative predictive value of ≥ 25% reduction in AUC_{0-20} after a single dose were respectively 84.6% and 50.0% (P = 0.146).

Conclusion
The protection provided by a single MLK-dose against EIB only modestly predicts the effect of regular treatment against EIB in adolescent asthmatics on low dose inhaled corticosteroids. If used on a daily base, MLK offered clinically significant protection against EIB in the large majority (80%) of children suboptimally controlled by low dose ICS.
INTRODUCTION

Asthma is a heterogeneous disease, which is reflected in the variability of individual patients’ responses to medications. It has been shown that symptomatic asthmatic children on low dose inhaled corticosteroids (ICS) show a considerable variability in response to the currently available step-up options: i.e., doubling the dose of ICS, adding a long-acting β2-agonist (LABA) or adding a leukotriene receptor antagonist (LTRA). There is little evidence to guide clinicians to the most effective step-up option.

Adding an LTRA to ICS to reinforce anti-inflammatory treatment is one of the step-up options in children with persistent asthma symptoms. Cysteinyl leukotrienes (CysLTs) are pro-inflammatory mediators causing potent and long-lasting airway narrowing. In exercise induced bronchoconstriction (EIB), CysLTs are released from activated mast cells as a result of airway drying and cooling. Compared to placebo, daily treatment with the LTRA montelukast (MLK) significantly attenuated EIB, measured by post-exercise maximum % fall in FEV₁ and area under the FEV₁ curve (AUC), in adults and in children uncontrolled by low dose ICS. In adult asthmatics, a single MLK-dose (10 mg) has been shown to provide a rapid (1h post-dosing) protection against post-exercise maximum % fall in FEV₁ compared to placebo. Other placebo controlled studies in both adults and children (aged 4-14y) showed a sustained protection 24h after a single MLK-dose (4, 5 or 10 mg). In these studies, the reduction in both maximum % fall in FEV₁ and AUC₀-60min was similar at 2h and 24h post-dosing.

However, MLK does not protect against EIB in all children and 20-40% of children are considered non-responders both after a single dose and longer-term daily treatment.

It is not clear if a single MLK-dose response against EIB relates to the clinical effectiveness following longer-term daily MLK-treatment within the same child. If both treatment responses are mediated through the same pathway, a single dose response should predict the longer-term clinical effectiveness of step-up therapy with MLK. In the present study, we investigated the relationship between a single dose response to MLK (5 or 10 mg) against EIB and the clinical effectiveness after 4 weeks once daily MLK-treatment against EIB in children with mild to moderate persistent asthma suboptimally controlled by low dose ICS.

METHODS

Subjects

Children were recruited from the pediatric outpatient clinic of the Medisch Spectrum Twente, Enschede, the Netherlands. Children with a clinical history of persistent asthma...
and EIB (confirmed by a previous exercise challenge), partly or fully uncontrolled by low stable doses of ICS alone (daily dose of 100 – 400 μg of beclomethasone dipropionate or equipotent) based on guideline derived symptom scores, were screened. Fifty-one children, aged 12-17 years, were screened by a standardized treadmill exercise challenge before starting treatment with MLK (5 or 10 mg depending on age). Children were included if a fall in FEV1 ≥10% from baseline occurred within 20 min post-exercise. Other inclusion criteria comprised the ability to perform reproducible pulmonary function tests (i.e., variation of percentage of the predicted value of FEV1 in 3 of 5 consecutive measurements < 5%) and baseline FEV1 ≥ 70% of predicted.

Exclusion criteria included viral upper airway infections, other lower airway or cardiac co-morbidities or hospitalization due to an asthma exacerbation in the month before inclusion. Furthermore, children were excluded for use of systemic corticosteroids, antihistamines, LTRA or anticholinergics in two weeks prior to the study or other medication changes during the treatment period. Children were not allowed to use short-acting bronchodilators within 8h or long-acting bronchodilators within 24h prior to testing or to perform vigorous exercise within 8h prior to testing.

The study was approved by the Medical Ethics Committee, Medisch Spectrum Twente, Enschede. All children gave written assent and their parents gave written informed consent. The study was registered online in the NTR register as NTR2059.

Study design
The study had a prospective, open-label design. During baseline visit, children performed an asthma control questionnaire (ACQ) and pediatric asthma quality of life questionnaire (PAQLQ) and were interviewed about their asthma symptoms, (rescue) medication use, allergies, smoking and other environmental factors. Children performed an exercise challenge with lung function measurements pre- and repeatedly up to 30 min post-challenge.

One week after the baseline visit, children were started on a therapeutic MLK-dose (5 or 10 mg QD, depending on their age) before bedtime. Twenty hours after the first dose (through of dosing interval) a second exercise challenge was performed. After 30 ± 4 days of treatment, a third exercise challenge was performed. Children received the last MLK-dose 40-44h prior to the third exercise challenge to ensure that the ‘longer-term’ anti-inflammatory effect was measured and not the more acute antagonistic effect of a single dose. Children were asked to bring their medication strip to the third visit to allow compliance check.

Spirometry
Pulmonary function tests were performed 5 min pre- and repeatedly post-exercise using a standardized protocol according to international guidelines. A calibrated Microloop
MK8 Spirometer (Micromedical, Quayside, United Kingdom) with Spida5® software was used to measure pulmonary volumes and flow-volume loops. All measurements were performed in duplicate and technically best values were included in the analysis. All spirometry values were expressed as percentage of the predicted value.

**Exercise challenge**

Exercise challenges were performed by running with nose clipped on a treadmill (Horizon® fitness Ti22, Cottage Grove, Wisconsin, United States) with an incline of 10% using a standardized ATS protocol. Exercise challenges in children have a good short term repeatability (mean difference in fall in FEV₁ -0.4%, 95% CI ± 12%). All exercise challenges were performed in the afternoon (between 1.30 and 5 p.m.). During exercise, children inhaled dry air with a temperature of 20 – 25 degrees Celcius and a humidity of 16 ppm. Heart rate was continuously monitored by a radiographic device (Polar Sport Tester®, Kempele, Finland) and the running speed of the treadmill was increased to raise the heart rate to approximately 90% of the predicted maximum. This speed was maintained for a total duration of 6 min. Spirometry was performed before exercise (baseline value) and 1, 3, 6, 9, 12, 15 and 20 min after exercise. Twenty minutes after exercise, or earlier at request, children received 100 µg salbutamol and spirometry was performed at \( t = 21, 23, 25 \) and 30 min until FEV₁ had returned to ≥ 95% of baseline value. If FEV₁ had not recovered to ≥ 95% of baseline after 30 min, children received a second dose of 100 µg salbutamol.

**Questionnaires**

The ACQ and PAQLQ questionnaires were performed at the screening and third visit to the outpatient clinic by the research assistant. The ACQ is used to assess asthma control and consists of 7 questions, scoring symptoms, daily rescue bronchodilator use and baseline FEV₁ % predicted. Children can respond to these questions on a 7-point scale. Baseline FEV₁ % predicted is scored by the lung function assistant. The questions weigh equally and the ACQ score is the mean and ranges between 0 (totally controlled) and 6 (severely uncontrolled).

The PAQLQ is used to measure quality of life and consists of 23 questions in 3 domains; symptoms, activity limitation and emotional function. Children can respond on a 7-point scale. The total PAQLQ score is the mean of all 23 questions and domain scores are the means of the items in those domains, ranging from 1 (impaired quality of life) to 7 (no impairment in quality of life).

**Statistical analysis**

Data consisted of 3 sets of pre-exercise and post-exercise FEV₁ values at pre-defined time points. Exercise induced bronchoconstriction was expressed as total area under
the curve from 0 to 20 min post-exercise (AUC$_{0-20\text{min}}$), calculated by a trapezoid rule, and post-exercise % fall in FEV$_1$ from baseline at each time point. The time to recovery to $\geq$ 95% of baseline FEV$_1$ was retained for analysis, and the percentage of children not recovered after 20 min was calculated. Children who received a dose of salbutamol before 20 min post-exercise were excluded from this analysis. If FEV$_1$ did not decrease below 95% of baseline, the time to recovery was assigned a value of zero.

As CysLTs produce a potent and longlasting bronchoconstrictor effect on airway smooth muscle, MLK was anticipated to mainly attenuate the duration of bronchoconstriction, measured by the AUC.$^3$ This was previously confirmed in several large adult studies demonstrating a greater reduction in post-exercise AUC than in the maximum post-exercise % fall in FEV$_1$ after regular treatment with MLK.$^{23-25}$ Hence, the primary end point was the correlation (reported as the intraclass correlation coefficient) between the change in AUC$_{0-20\text{min}}$ after a single MLK-dose and after 4 weeks MLK-treatment. Secondary end points were the correlation between change in maximum % fall in FEV$_1$ after a single MLK-dose and 4 weeks MLK-treatment and percentage protection against EIB (defined as % reduction in AUC$_{0-20\text{min}}$ and maximum% fall in FEV$_1$) provided by a single MLK-dose and 4 weeks MLK-treatment. Changes between screening, second and third visits in all outcome variables were analyzed with Students’ paired t-test (for normally distributed variables, i.e., FEV$_1$ values and % fall in FEV$_1$) or Wilcoxon-signed rank test (for variables with a skewed distribution, i.e., ACQ and PAQLQ).

Children with $<25\%$ reduction in AUC$_{0-20\text{min}}$ after MLK were considered non-responders. A cross-tabulation was made of responders and non-responders to a single MLK-dose and 4 weeks MLK-treatment. Fisher’s exact test was used to calculate the positive and negative predictive value of a single dose response.

SPSS$^*$ 20.0 for Windows$^*$ was used for statistical analysis. A $P$-value of $< 0.05$ was considered statistically significant. The sample size estimated to detect a difference of -0.60 between the null hypothesis correlation of 0.00 and the alternative hypothesis correlation of 0.60 using a two-sided hypothesis test with a significance level of 5% and 80% power, was 19 subjects.

**RESULTS**

Fifty-one children were screened by a standardized treadmill exercise challenge. Twenty-seven children had a $<10\%$ post-exercise fall in FEV$_1$ and were excluded. Twenty-four eligible children were included of whom twenty-one completed the study. Patient baseline characteristics are presented in Table 1. One girl was excluded because she underwent another medication change during the treatment period and two other children dropped out for reasons unrelated to MLK-treatment or the study protocol.
Three children reported adverse events during the treatment period with MLK. Two of them complained of headache during the first days of treatment, and one child reported an increase in pre-existing symptoms of dizziness. All adverse events were mild and self-limiting.

**Table 1.** Baseline characteristics of study population (n = 24)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>45.8%</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>14.4 ± 1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>55.8 ± 13.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 12.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.8 ± 3.1</td>
</tr>
<tr>
<td>Baseline FEV₁ (% pred.)</td>
<td>92.9 ± 12.4</td>
</tr>
<tr>
<td>Allergic</td>
<td>86%</td>
</tr>
<tr>
<td>RAST animal dander positive</td>
<td>54%</td>
</tr>
<tr>
<td>RAST house dust mite positive</td>
<td>75%</td>
</tr>
<tr>
<td>RAST tree pollen positive</td>
<td>46%</td>
</tr>
<tr>
<td>RAST grass pollen positive</td>
<td>46%</td>
</tr>
<tr>
<td>nasal corticosteroid use</td>
<td>50%</td>
</tr>
<tr>
<td>LABA use</td>
<td>13%</td>
</tr>
<tr>
<td>ICS use</td>
<td>100%</td>
</tr>
<tr>
<td>ICS daily dose (µg)</td>
<td>200 (100 – 400)</td>
</tr>
</tbody>
</table>

*Data expressed as mean ±SD, median (range) or percentage of total patients. BMI = body mass index, RAST = radio-allergosorbent test, LABA = long acting β2-agonist, SABA = short acting β2-agonist, ICS = inhaled corticosteroid.*

All children included in this study were clinically suboptimally controlled on low dose ICS (stable dose for at least 6 weeks) and used short-acting β2-agonists on an as-needed basis, with a self-reported mean (± SD) use of 1.9 (± 1.8) puffs per week before the baseline exercise challenge and 1.6 (± 1.6) puffs per week after 4 weeks of treatment with MLK. FEV₁ after a single MLK-dose and after 4 weeks treatment were not significantly different from FEV₁ before the baseline exercise challenge. All outcome variables are summarized in Table 2.

**Exercise induced bronchoconstriction**

Exercise induced bronchoconstriction, expressed as the AUC₀-20min, decreased significantly after a single MLK-dose (P = 0.001, CI 64.9 – 218.2), but not after 4 weeks of treatment (P = 0.080, CI -12.2 – 200.4). There was a moderate correlation between the response to a single MLK-dose and 4 weeks MLK-treatment on exercise-induced AUC₀-20min, r = 0.49 (P = 0.011) (Fig. 2A).
<table>
<thead>
<tr>
<th>End Point</th>
<th>baseline</th>
<th>single dose</th>
<th>difference (95% CI)</th>
<th>P-value</th>
<th>4 weeks</th>
<th>difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$\text{0-20min}$ (%.min)</td>
<td>309.4 ± 221.3</td>
<td>167.9 ± 153.0</td>
<td>141.5 (64.9-218.2)</td>
<td>&lt;0.05</td>
<td>215.3 ± 208.7</td>
<td>94.1 (-12.2-200.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Maximum % fall in FEV$_1$</td>
<td>24.1 ± 13.4</td>
<td>18.3 ± 11.7</td>
<td>5.8 (2.4-9.2)</td>
<td>&lt;0.05</td>
<td>19.7 ± 15.0</td>
<td>4.5 (-1.9-10.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Recovery time (min)</td>
<td>21 (3-30)</td>
<td>12 (0-23)</td>
<td>9</td>
<td>&lt;0.05</td>
<td>15 (0-21)</td>
<td>6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Baseline FEV$_1$ (% pred.)</td>
<td>92.3 ± 11.9</td>
<td>92.5 ± 11.9</td>
<td>-0.2 (-2.9-2.5)</td>
<td>0.89</td>
<td>91.4 ± 12.6</td>
<td>0.9 (-1.9-3.8)</td>
<td>0.52</td>
</tr>
<tr>
<td>Weekly SABA use (puffs)</td>
<td>1.9 ± 1.8</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>1.6 ± 1.6</td>
<td>0.3 (-0.3-0.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>ACQ score</td>
<td>1.07 ± 0.83</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.96 ± 0.73</td>
<td>0.11 (-0.25-0.46)</td>
<td>0.53</td>
</tr>
<tr>
<td>PAQLQ total</td>
<td>6.04 ± 0.98</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>6.34 ± 0.54</td>
<td>-0.31 (-0.71-0.09)</td>
<td>0.13</td>
</tr>
<tr>
<td>PAQLQ symptoms</td>
<td>5.82 ± 1.13</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>6.10 ± 0.72</td>
<td>-0.28 (-0.79-0.24)</td>
<td>0.28</td>
</tr>
<tr>
<td>PAQLQ activity limitation</td>
<td>5.66 ± 1.11</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>6.13 ± 0.66</td>
<td>-0.48 (-0.93-0.02)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PAQLQ emotional function</td>
<td>6.54 ± 0.99</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>6.79 ± 0.54</td>
<td>-0.24 (-0.56-0.07)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SD or median (range), as appropriate. AUC$\text{0-20min}$ = Area Under the Curve 0-20 min post-exercise, SABA = short acting β2-agonist, ACQ = asthma control questionnaire, PAQLQ = pediatric asthma quality of life questionnaire.
Exercise-induced maximum % fall in FEV₁ decreased significantly after a single MLK-dose ($P = 0.002$, CI 2.4 – 9.2), but not after 4 weeks of MLK-treatment ($P = 0.16$, CI -1.9 – 10.9). There was however no significant difference in maximum % fall in FEV₁ between a single MLK-dose and 4 weeks of MLK-treatment. Mean % fall in FEV₁ (± SEM) at each time point post-exercise is shown in Fig. 1 for all three exercise challenges. There was a weak correlation between the response to a single MLK-dose and to 4 weeks MLK-treatment on exercise induced maximum % fall in FEV₁, $r = 0.40$ ($P = 0.035$) (Fig. 2B).

A single dose of MLK and 4 weeks MLK-treatment respectively provided 45.7% vs. 30.4% reduction in AUC$_{0-20\text{min}}$ and 24.0% vs. 18.5% reduction in maximum % fall in FEV₁. When children with ≥ 25% reduction in AUC$_{0-20\text{min}}$ were considered responders to MLK, 13 out of 21 (62%) could be considered responders after a single MLK-dose and 15 out of 21 (71%) after 4 weeks of MLK-treatment. Table 3 shows a cross-tabulation of responders and non-responders based on reduction in AUC$_{0-20\text{min}}$. In this study, the positive predictive value of a single MLK-dose response was 84.6% and the negative predictive value 50.0% ($P = 0.146$).

Complete data sets for the evaluation of recovery were available for 18 children, as 3 children received a rescue gift of salbutamol before 20 min post-exercise (1 child after the baseline exercise challenge, 2 children after the second challenge and 2 children after the third challenge). After the screening exercise challenge, 35% of children recovered.
Fig. 2. Change in $AUC_{0-20min}$ (panel A) and maximum % fall in FEV$_1$ (panel B) between a baseline exercise challenge test (ECT) and an ECT after a single MLK-dose and between a baseline ECT and an ECT after 4 weeks MLK-treatment.

$AUC_{0-20min} =$ area under the curve, FEV$_1 =$ forced expiratory volume in 1s, MLK = montelukast
to ≥ 95% of baseline within 20 min; after a single MLK-dose and 4 weeks MLK-treatment resp. 68% and 72% of children recovered within 20 min. However, these differences were not statistically significant.

Table 3. Cross-tabulation of the response to montelukast

<table>
<thead>
<tr>
<th>Response after single dose</th>
<th>Response after 4 weeks treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td>Responder</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Non-responder</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>13</td>
</tr>
<tr>
<td>Non-responder</td>
<td>Responder</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Non-responder</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

A responder is defined as ≥ 25% reduction in AUC$_{0-20min}$ compared to baseline AUC$_{0-20min}$.

Quality of Life and Asthma Control

At the baseline visit children had a mean ACQ score of 1.07 ± 0.83 units. Based on their ACQ, 10 out of 21 children (48%) could be considered partly or fully uncontrolled (ACQ score ≥ 0.75), although all children were suboptimally controlled based on guideline derived symptom scores². After 4 weeks MLK-treatment there was no change in mean ACQ scores ($P = 0.53$, CI -0.25 – 0.46) and 8 out of 21 children (38%) could still be considered partly or fully uncontrolled.

At baseline, quality of life was slightly impaired with a mean PAQLQ score of 6.04 ± 0.98 units. After 4 weeks MLK-treatment there was no significant difference in total PAQLQ score ($P = 0.13$, CI -0.71 – 0.09). However, there was a small, but significant improvement in the PAQLQ activity limitation domain score ($P = 0.040$, CI -0.93 to -0.02).

DISCUSSION

In this study we found a moderate correlation between the protective effect against EIB of a single MLK-dose and 4 weeks of MLK-treatment, expressed as post-exercise AUC$_{0-20min}$ and maximum % fall in FEV$_1$. A single MLK-dose provided a greater reduction in AUC$_{0-20min}$ post-exercise (45.7%) than 4 weeks of MLK-treatment (30.4%). A minority of the children (19.0%) failed to show protection against EIB after either a single MLK-dose or 4 weeks of MLK-treatment.

This was the first study that separated the single dose and longer term response to MLK by assessing EIB 20-24h after a single MLK-dose, and 40-44h after 4 weeks MLK-treatment, to measure their relationship. Previous studies both in adults¹¹,¹² and children¹³ showed a similar attenuation in EIB performed at the through interval (24h) after a single MLK-dose. Bronsky et al.²⁶ observed in adult asthmatics that this protective effect expired 32-36h after two once daily doses of MLK (2, 10 or 50 mg). However, Kim et
al. found a prolonged protective MLK-effect against EIB in asthmatic children, i.e. 48h after the last dose of 8 weeks daily treatment, suggestive of anti-inflammatory properties of regular MLK-treatment. In an animal model of allergic asthma, it was shown that 4 weeks treatment with MLK has anti-inflammatory effects on the airway wall and lung parenchyma. In all other previous pediatric studies describing longer-term protection against EIB, children performed an exercise challenge at the end of the dosing interval (i.e., 20-24h post-dosing), which is expected to reflect the composite response to both the more acute, functional antagonistic and the long(er)-term, anti-inflammatory properties of MLK. By measuring EIB 40-44h after the last dose of 4 weeks of regular MLK-treatment we allowed assessment of the anti-inflammatory MLK-effect only.

Our data can be affected by several factors. Firstly, the timing of the exercise challenges may have influenced our data. Based on pharmacological data showing a plasma half life of MLK of 2.7-5.5h, measuring the effect of a single MLK-dose 20-24h after dosing may underestimate the acute antagonistic effect of MLK. However, previous studies in both adults and children (aged 4-14y) showed that the reduction in both maximum % fall in FEV₁ and AUC₀₋₆₀₅₈ was similar at 2h and 24h after a single MLK-dose.

The 40-44h time interval between the last dose of MLK and the exercise challenge can explain why we found a smaller reduction in AUC (30.5%) and maximum % fall in FEV₁ (18.5%) after 4 weeks of MLK-treatment. Previous pediatric studies reported 63.8% reduction in AUC and 44.8 - 56.5% reduction in maximum % fall in FEV₁, but measured the effect 10-24h after the last dose. However, the small effect of MLK on EIB after 4 weeks was consistent with the lack of effect on ACQ and PAQLQ scores. The treatment period of 4 weeks might have been rather short to evaluate the anti-inflammatory effects of MLK, but was similar to other pediatric studies describing the effect of regular daily MLK on EIB.

A second possible confounder is the lack of a placebo arm. This study was designed to correlate the single dose response to MLK and the response to regular daily MLK-treatment. Both have been shown to be effective against EIB in asthmatic children in previous placebo controlled studies. As the children included in this study were partly or fully uncontrolled by low dose ICS alone, we decided not to withhold proper add-on treatment any longer than necessary.

A third factor that may have influenced our results is that based on ACQ scores both well controlled, as well as fully uncontrolled children were included. However, based on clinical symptoms, all children were partly or fully uncontrolled according to international guidelines. Furthermore, all children had ≥ 10% fall in FEV₁ at baseline, which is a sign of uncontrolled asthma. Subgroup analysis comparing children with ACQ scores <0.75 vs. ≥0.75 did not show any significant differences.
Furthermore, children took MLK at home, unsupervised by hospital staff. We explicitly instructed the children and their parents about the timing of the medication gifts, and checked their compliance by checking their medication strip during the study and following study completion. Asthmatic children in our outpatient clinic are reviewed for their adherence and inhalation technique every three months.

Interestingly, in our study a single dose provided a greater mean protection against EIB than 4 weeks treatment. However, as some children reached a significant attenuation of EIB only after 4 weeks of treatment, or showed greater protection against EIB after 4 weeks of MLK-treatment, the absolute number of responders was greater after 4 weeks of treatment. Meanwhile, some patients only responded to a single MLK-dose and not to 4 weeks treatment. We speculate that this heterogeneity in response to a single MLK-dose and 4 weeks of MLK-treatment reflects the variable progression of airway inflammation and remodeling in children with asthma. When inflammation is relatively mild and EIB is mainly the result of transient increased airway smooth muscle tone due to mediator release, a single dose of MLK is effective and inflammation is easily reversible within 4 weeks treatment. However, when inflammation is more progressed and airway remodeling more pronounced, 4 weeks daily treatment is probably insufficient. In these patients, EIB might bounce back after the single dose effect of MLK has waned.

In real life, children using step-up treatment with MLK on a daily base will benefit from both the single dose antagonistic effect and the anti-inflammatory effect of regular treatment. In our study, only 4 (19%) of children could be considered true non-responders with no response to either a single dose or 4 weeks treatment. Previous studies found similar or higher percentages of non-responders (ranging from 17.9% - 43%) based on the reduction in % fall in FEV\textsubscript{1} after regular treatment with MLK. However, these studies used a variety of different cut-off values to differentiate between responders and non-responders. Some studies considered patients with ≥ 50\%\textsuperscript{5} reduction in maximum % fall in FEV\textsubscript{1} responders; others considered all patients with < 10\%\textsuperscript{6}, < 20\%\textsuperscript{6,16,24,25} or < 30\%\textsuperscript{23} fall in FEV\textsubscript{1} responders. We chose to define MLK-responders based on AUC, as large studies both in adults\textsuperscript{23-25} and children\textsuperscript{6} have shown that following exercise challenge, MLK has a greater impact on AUC than on maximum % fall in FEV\textsubscript{1}. For children practicing sports, the AUC, representing both the severity and duration of EIB, is at least as important as the maximum % fall in FEV\textsubscript{1}.

Montelukast has been shown to provide similar or better protection against EIB after 4 weeks treatment compared to other step-up options. Long-acting β2-agonists initially provide significant protection against EIB, but as tolerance develops, the bronchoprotective effect of long-acting β2-agonists wanes after 4 weeks treatment.\textsuperscript{4,7,28} Doubling
the dose of ICS did not result in significant differences in EIB after 3 weeks treatment\textsuperscript{29,30}, and quadrupling the dose of ICS resulted in only 26.1 - 34.8\% reduction in AUC\textsuperscript{30}.

In conclusion, in the present study we found that the protection provided by a single dose of MLK against EIB only modestly predicts the effect of regular MLK-treatment against EIB. The single dose response to MLK was stronger than the response to 4 weeks regular treatment, implying that a high adherence is essential to profit from the full protective effect of MLK against EIB. If used on a daily base, MLK offered clinically significant protection against EIB in the large majority (80\%) of children suboptimally controlled by low dose ICS.
REFERENCES


