

A long-term clinical trial on the efficacy and safety profile of doxofylline in Asthma: The LESDA study



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ABSTRACT

Doxofylline, an oral methylxanthine with bronchodilator and anti-inflammatory activities, offers a promising alternative to theophylline due to its superior efficacy/safety profile. No long-term studies on the efficacy and safety of doxofylline are currently available in asthma. The aim of the Long-term clinical trial on the Efficacy and Safety profile of Doxofylline in Asthma (LESDA) study was to investigate the safety and efficacy profile of doxofylline administered for one year in asthmatic patients. LESDA was a multicenter, open-label, Phase III, clinical trial in which adult asthmatic patients received the same treatment (oral doxofylline 400 mg t.i.d.) for one year. Efficacy was assessed through periodic pulmonary function tests and by having the subjects keep monthly records of asthma events rates and use of salbutamol as rescue medication. The rate of adverse events (AEs) was recorded during the study.

Three-hundred nine patients were screened and allocated in the study. Doxofylline significantly improved the change from baseline in forced expiratory volume in 1 s (FEV₁) (+16.90 ± 1.81%, *P* < 0.001 vs. baseline). Doxofylline also significantly improved the rate of asthma events (events/day: -0.57 ± 0.18, *P* < 0.05 vs. baseline) and the use of salbutamol as rescue medication (puffs/day: -1.48 ± 0.25, *P* < 0.01 vs. baseline). The most common AEs were nausea (14.56%), headache (14.24%), insomnia (10.68%), and dyspepsia (10.03%). There were neither serious AEs nor deaths during or shortly after the study. Concluding, doxofylline is effective and well tolerated when administered chronically in asthmatic patients.

1. Introduction

Doxofylline is an orally active "novofylline" belonging to the class of methylxanthine that is characterized by both anti-inflammatory and bronchodilator activities [1]. Doxofylline has shown similar efficacy to theophylline in asthmatic patients but with significantly fewer side effects [2].

The anti-inflammatory activity of doxofylline has been confirmed *in vitro* in human monocytes treated with phorbol 12-myristate 13-acetate or lipopolysaccharide [3], airway smooth muscle (ASM) cells, pre-clinical studies by using murine models of allergic and a non-allergic lung inflammation [4–6], and in patients with chronic bronchitis [7]. Doxofylline is also effective in preventing the ASM contractile response induced by platelet-activating factor and methacholine in experimental

animals [8,9].

The pooled analysis of two double-blind, randomized, placebo-controlled trials performed in asthmatic patients and lasting 12 weeks that investigated the impact of Doxofylline compared to Theophylline, the DOROTHEO 1 and 2 studies, showed that doxofylline offers a promising alternative to theophylline with a superior efficacy/safety profile in the management of patients with asthma [10].

Unlike other methylxanthines such as theophylline, doxofylline does not modulate the activity of certain cellular receptors/enzymes such as adenosine receptors except for A_{2A} receptor subtype, phosphodiesterases (PDEs) except for PDE_{2A1} isoform, and histone deacetylase enzymes, and does not alter the movement of calcium into cells [11]. These specific characteristics may account for the favourable safety profile of doxofylline.

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In any case, to date long-term studies on the efficacy and safety of doxofylline are still missing in asthma. Therefore, the Long-term clinical trial on the Efficacy and Safety profile of Doxofylline in Asthma (LESDA) was performed to investigate the impact of doxofylline administered for one year in the treatment of subjects with asthma.

2. Materials and methods

2.1. Study design

LESDA was a Phase III, multicenter, open-label, single-arm, clinical trial conducted in 13 centres in the US [12]. The study had one-week run-in period during which the subject took salbutamol as needed followed by 52-week treatment period and a 1-week run-out phase at the end of the study. All patients received doxofylline 400 mg administered orally with immediate release formulations and three times daily (t.i.d.) during the period of study. Doxofylline 1.200 mg/day (400 mg t.i.d.) is the highest approved dose in adult asthmatic patients [13–15]. The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation/Good Clinical Practice Guidelines and local regulations. The study protocol was reviewed and approved by Institutional Review Boards at each study centre. The study has been registered in the International Standard Randomised Controlled Trial Number (ISRCTN10030693) and detailed information can be found at <http://www.isrctn.com/ISRCTN10030693>.

2.2. Study population

Patients with asthma who were ≥ 18 years old and were non-smokers for at least 6 months before entering the study, who had forced expiratory volume in 1 s (FEV_1) within 50%–80% of the predicted and who showed at least 15% post-bronchodilator (salbutamol 2 puffs, 200 μ g) increase in FEV_1 were enrolled. For safety reasons, the reversibility test was conducted with the salbutamol dosage at 200 μ g in order to prevent adverse events (AEs) related to the administration of β_2 -adrenoceptor (AR) agonists, such as tachycardia or hand tremor [16]. The use of inhaled corticosteroid (ICS) and/or cromolyn sodium was permitted if the subject had been taking a stable dose for at least 30 days. If ICS and/or cromolyn sodium was required during the study, it was recommended that a doxofylline baseline level be achieved (minimum, 14 days) before the administration of any additional medications.

Key exclusion criteria included serious concomitant cardiovascular, neurologic, gastrointestinal, renal, hepatic, hematologic, metabolic diseases, and chronic obstructive pulmonary disease (COPD). Subjects using oral steroids or oral β -agonists were excluded, as well as pregnant or lactating women. Patients that had participated in other investigational drug studies within 30 days before the start of this study were excluded, except the DOROTHEO 1 and 2 trials [10] that preceded this study. The study flowchart is reported in supplementary data file (Table S1).

Subjects were required to refrain from the use of theophylline and aminophylline. Patients were permitted to use inhaled salbutamol as rescue medication. Additional medication of any type that the physician gave to a subject was recorded on the case report form. The use of salbutamol as rescue medication within 8 h of a scheduled pulmonary function test was prohibited. If a subject needed to take salbutamol within this time, the study visit was to be rescheduled as late as noon of that day to accommodate 8-h prohibition.

2.3. Study endpoints

The primary endpoint of this study was the change from baseline in FEV_1 , expressed as percentage (%), after 52 weeks of treatment.

The secondary endpoints included: i) the overall change from baseline in FEV_1 during the study period; ii) the change from baseline in

asthma events rate (n/day; asthma events were defined as the increased symptoms or increased airflow limitation requiring the use of as needed bronchodilators [17]) after 52 weeks of treatment and the overall change from baseline during the study period; and iii) the change from baseline in salbutamol use rate (puffs/day) after 52 weeks of treatment and the overall change from baseline during the study period.

The patients used diary cards to record the date and time that each dose of study medication was taken, date and time of asthma episodes, date and time of each salbutamol use, and date and time of any AE.

2.4. Assessment of safety and drop-outs endpoints

All clinical AEs were recorded and graded as mild, moderate or severe. Their relationship with the treatment was classified as follows: 1) not related, 2) possibly related, 3) definitely related or 4) unknown. The duration of the symptoms and the action taken (none, reduction of the dose or discontinuation of treatment) were also recorded for each AE. Subjects were removed from the therapy or assessment for: 1) unacceptable deterioration of clinical state, 2) non-adherence to treatment, 3) persistent drug-related AEs with patient's willingness to discontinue treatment, 4) occurrence of pregnancy during the study.

2.5. Statistical analysis

Data are expressed as mean \pm standard error of the mean (SEM). The statistical analysis compared FEV_1 measured by spirometry obtained at baseline (immediately prior to the start of treatment) with the results obtained 2 h after administration of study medication (2-h postdose FEV_1) at each visit during the study period. The derived variable was the percent change between these two assessments. Absolute changes were calculated for the asthma events rate (total number of events divided by total number of days on study medication) and salbutamol use rate (total number of puffs divided by total number of days on study medication). Baseline for the latter two variables was defined as the value obtained from the diaries during the run-in phase. The safety analysis was performed by calculating the frequency of AEs in the study population.

The correlation analysis between the change from baseline in FEV_1 , asthma events, salbutamol use rate induced by doxofylline and the serum levels of doxofylline was performed by using a Pearson test and expressed as linear regression with 95% confidence bands.

The analysis of the changes from baseline was performed by using *t*-test. All differences were considered significant for $P < 0.05$. Data analysis was performed by using Prism 5 software (GraphPad Software Inc, CA, USA).

3. Results

3.1. Patients population

Three hundred nine patients were screened and enrolled in this study. Patient enrolment and the reasons for discontinuation are presented in Fig. 1, the dataset analyzed is shown in Table S2 and the number of patients at each visit in Table S3. The patient demographics and baseline characteristics are shown in Table 1, and the summary of prior asthma medications is reported in Table S4.

3.2. Lung function (FEV_1)

Doxofylline 400 mg significantly ($P < 0.001$) increased 2-h post-dose FEV_1 compared to baseline during the 52 weeks of treatment. The improvement in 2-h postdose FEV_1 remained consistently above the minimal clinically important difference (MCID) of 12% and 200 mL (overall increase: $+16.90 \pm 1.81\%$, $+390.30 \pm 41.90$ mL, both $P < 0.001$ vs. baseline) during the study period (Fig. 2A and B).

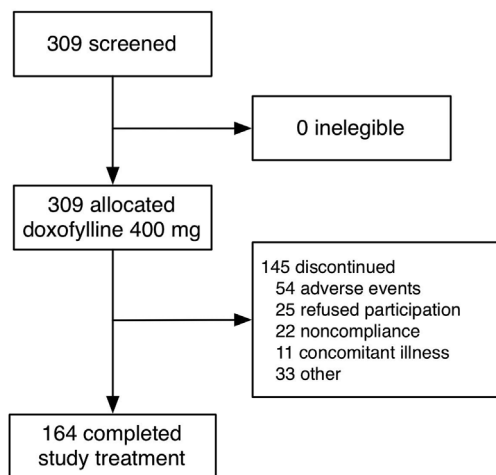


Fig. 1. CONSORT diagram of patient flow in the LESDA study.

Table 1

Summary of demographic and baseline data of patients enrolled in the LESDA study.

	Doxofylline 400 mg
Number of subjects	309
Age (yr)	
Mean (SEM)	38.2 (0.7)
Sex	
Male	136 (44.0%)
Female	173 (56.0%)
Race	
Caucasian	251 (81.2%)
Black	37 (12.0%)
Hispanic	18 (5.8%)
Other	3 (1.0%)
Body weight (kg)	
Mean (SEM)	81.35 (1.08)
Height (cm)	
Mean (SEM)	167.66 (0.63)
FEV ₁ , L	
Mean (SEM)	2.31 (0.02)
% of predicted FEV ₁	
Mean (SEM)	65.4 (0.5)
No. of asthma events/day	
Mean (SEM)	1.87 (0.16)
Salbutamol use (puffs/day)	
Mean (SEM)	3.61 (0.33)
Precipitating factors	
Yes	297 (96.1%)
No	12 (3.9%)
Hospitalizations for asthma	
Yes	131 (42.8%)
No	178 (57.6%)
Age at onset of asthma (yr)	
Mean (SEM)	18.70 (1.00)
Years since onset	
Mean (SEM)	19.50 (0.70)

FEV₁ = forced expiratory volume in 1 s.

SEM = standard error of mean.

3.3. Asthma events and rescue medication

Doxofylline 400 mg significantly reduced the rate of asthma events compared to baseline after 52 weeks of treatment (events/day: -0.57 ± 0.18 , $P < 0.05$ vs. baseline; Fig. 3A). Doxofylline 400 mg also significantly improved the change from baseline in the use of salbutamol as rescue medication during the study period (puffs/day: -1.48 ± 0.25 , $P < 0.01$ vs. baseline; Fig. 3B).

3.4. Correlation between efficacy results and serum levels of doxofylline

No significant ($P > 0.05$) correlation was detected between the improvement in FEV₁ or asthma events with the serum levels of doxofylline (Fig. 4A and B). Conversely, significant linear correlation (Pearson: $r = -0.87$, $P < 0.05$; linear regression: slope -0.15 ± 0.05 , $R^2 = 0.75$) resulted between the serum levels of doxofylline and the reduction in the use of salbutamol as rescue medication (Fig. 4C).

3.5. Safety profile

Table 2 shows the frequency of AEs occurred in $\geq 2\%$ patients treated with doxofylline 400 mg during the study period. The most reported AEs were nausea (14.56%), headache (14.24%), insomnia (10.68%), and dyspepsia (10.03%). Overall, the AEs were mild or moderate in severity and generally well tolerated.

AEs were among the reasons for the withdrawal of 17.48% patients from the study. The AEs most commonly leading to discontinuation were nausea, headache, and insomnia, and usually occurred within the first month of treatment.

No subjects experienced serious AEs. No subjects died during or shortly after finishing the study.

4. Discussion

This long-term, multicenter, clinical trial met the primary and both secondary endpoints. Generally, doxofylline 400 mg significantly improved pulmonary function and disease control in subjects with asthma.

After one year of treatment, the change from baseline in FEV₁ elicited by doxofylline 400 mg was $\approx 16\%$, together with concomitant reduction in the rate of asthma events and use of salbutamol as rescue medication. As expected, the increase in FEV₁ induced by doxofylline 400 mg resulted significant already at the first study time-point, after 4 weeks of treatment, and interestingly it remained constantly greater than the MCID (increase in FEV₁ $\geq 12\%$ and ≥ 200 mL) during all the 52 weeks of treatment [18,19]. Analogously, also the use of salbutamol as rescue medication was reduced in a significant manner after just 4 weeks of treatment, and it further improved during the study period. On the other hand, although the treatment with doxofylline 400 mg produced an appreciable reduction in the rate of asthma events after 4 weeks of treatment, such an improvement became significant from the second month until the end of the study.

Although the LESDA study was a long-term clinical trial, its results were generally similar in magnitude to those seen in the earlier comparative studies DOROTHEO 1 and 2 [10], that investigated the impact of doxofylline and theophylline in asthmatic patients for a shorter period of time of 12 weeks. This is an important consideration suggesting that the LESDA study was generally free from the main matters that arise in clinical trials with long-term follow-up and that could have introduced bias in the causal effect of a pharmacological intervention, such as the non-compliance, treatment switching, loss to follow-up, and truncation by death and other events [20].

The percentage of patients with atopic asthma was not recorded at screening. However, considering the normal distribution of baseline characteristics of patients enrolled in the LESDA study, including the age at onset of asthma, and that childhood-onset disease (0–11 yr) is mainly associated with atopic asthma [21–23], it can be estimated with good approximation that $\approx 23\%$ of subjects were affected by atopic asthma [24]. This may explain the further reduction in asthma events and use of salbutamol as rescue medication at around 26 and 30 weeks of treatment, corresponding to late spring-early summer season during the study period. This trend is consistent with the clinical improvement in patients suffering from difficult-to-control asthma during such a change of season [25,26], especially in inner city subjects living in US where the LESDA study was performed [27,28]. In this respect, looking at the frequency of asthma events and salbutamol use, and the

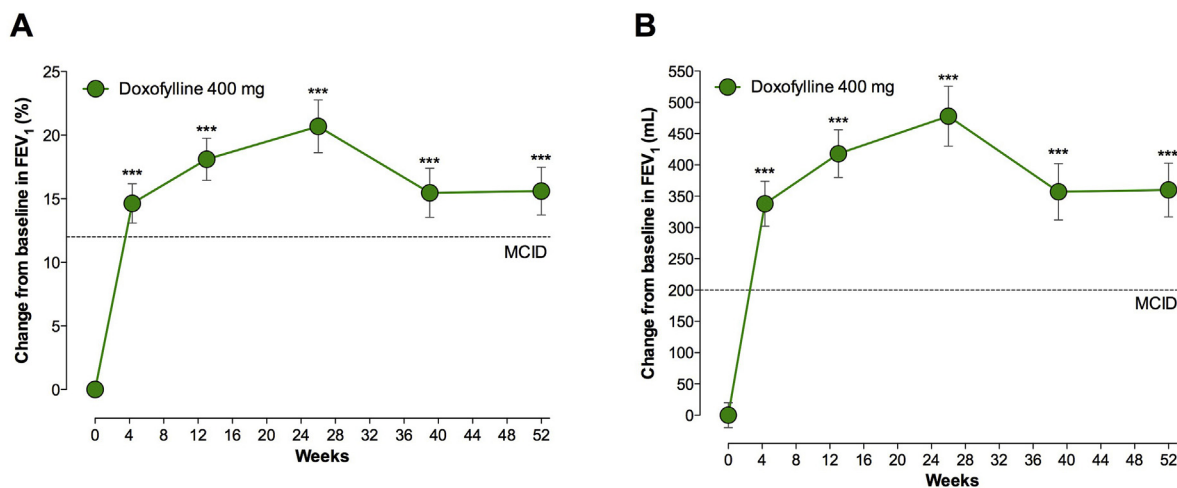


Fig. 2. Impact of doxofylline 400 mg on the change from baseline in 2-h postdose FEV₁ (A: %; B: mL), as measured during clinic visits. FEV₁: forced expiratory volume in 1 s; MCID: minimum clinically important difference. ***P < 0.001 vs. baseline (T0; statistical analysis assessed via t-test).

percentage of patients with precipitating factors and hospitalizations for asthma at baseline, it is evident that the population enrolled in this study was prevalently characterized by patients with poorly controlled asthma [29].

Interestingly, the dose of doxofylline used in this study was consistent with the approval documents and manufacturers recommendations. In fact, although doxofylline 400 mg sustained release tablet once daily or 400 mg tablet b.i.d. can be administered in adult patients with asthma, doses as high as 1.200 mg/day (400 mg t.i.d.) may also be prescribed on the basis of the clinical response and according to disease severity [13–15].

After 26 weeks of treatment there was a reduction in the efficacy of doxofylline on FEV₁ improvement. The mechanisms leading to the bronchodilator and an anti-inflammatory effect of doxofylline are not fully understood, however it has been reported in vitro that this drug may elicit ~50% inhibition of adenosine A_{2A} subtype receptor and PDE_{2A1} isoform activity at concentrations that are likely to be achieved in patients following oral dosing [30]. Therefore, it cannot be excluded that chronic treatment with doxofylline may induce some form of tolerance on the pathways modulated by these targets, as previously reported [31–33]. In any case, the reduction in the efficacy on FEV₁ after repeated dosing of doxofylline was not clinically relevant [18,19].

The baseline characteristics of the enrolled patients also justify the main weakness of this study, that is represented by the lack of a placebo arm. In fact the Ethics Committee [12] found unethical treating poorly controlled asthmatic patients with placebo in the light of the positive results obtained by the previous multicentre, double-blind, randomized trials that investigated the impact of doxofylline compared to theophylline in asthma, the DOROTHEO 1 and 2 studies [34,35]. In any case, despite the lack of a placebo arm, the findings obtained in the LESDA study generally confirm those of DOROTHEO 1 and 2 trials. In other words, the effect induced by 12 weeks of treatment with doxofylline [10] on lung function, frequency of asthma events and use of salbutamol as rescue medication were maintained during the whole 52 weeks of treatment of the LESDA study.

A further apparent limitation of the LESDA study is that the data of this trial [12], along with those of DOROTHEO 1 and 2 studies [34,35], have been made publically available many years after their conclusion. Indeed this is unusual, however we cannot omit that such a delay in the data publication was related with proven licensing matters across the pharmaceutical companies involved in the research and development of doxofylline in chronic obstructive respiratory disorders [36]. Fortunately, recently these controversies have been solved, thus permitting to register the trials in the ISRCTN and update the results in agreement

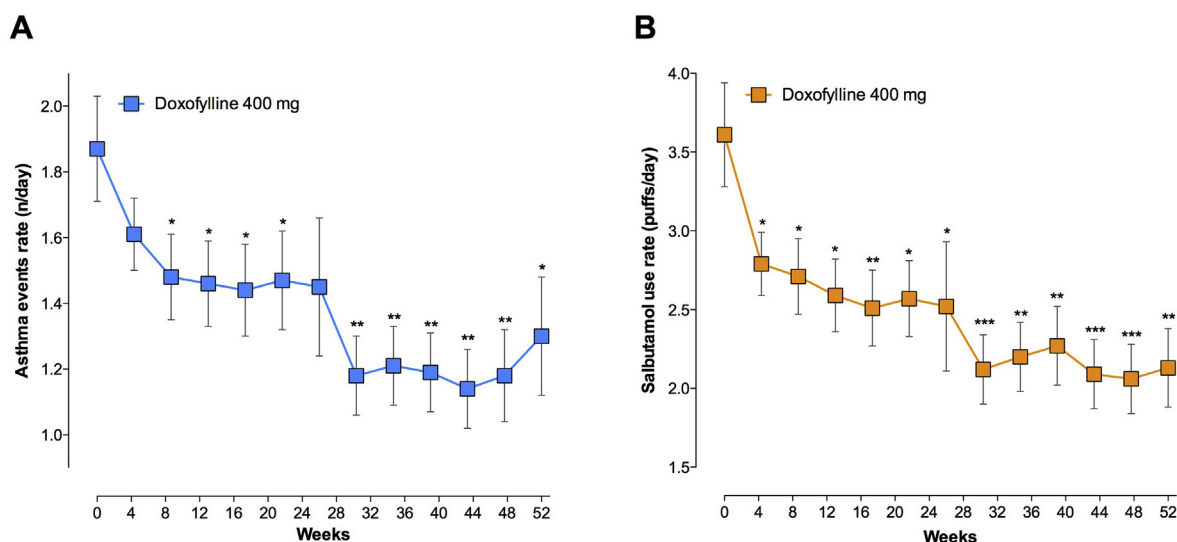


Fig. 3. Impact of doxofylline 400 mg on asthma events rate (events/day, A) and salbutamol use rate (puffs/day, B), as measured during clinic visits. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. baseline (T0; statistical analysis assessed via t-test).

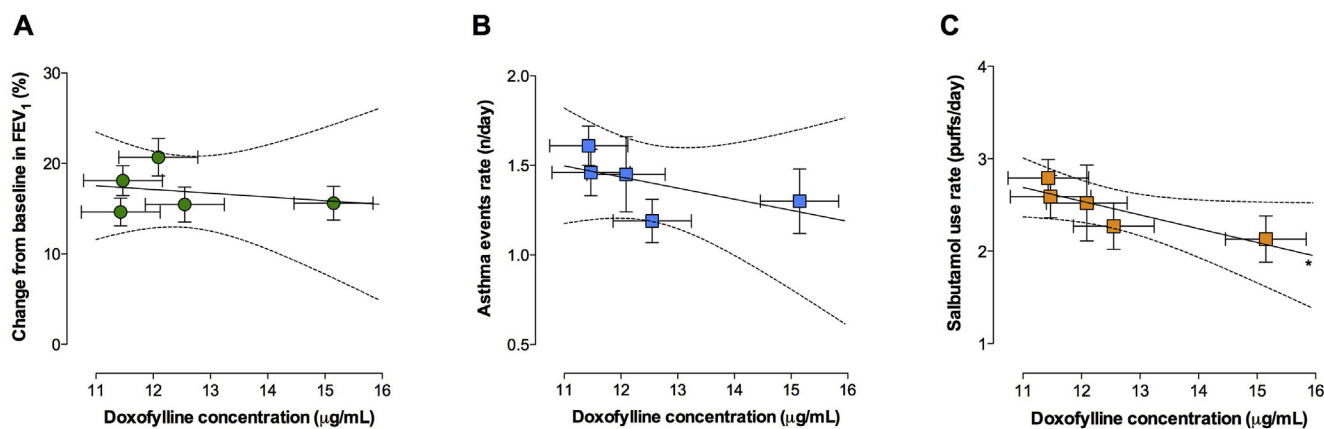


Fig. 4. Linear correlation by Pearson analysis with 95% confidence bands between efficacy outcomes and serum levels of doxofylline (change from baseline in FEV₁ [%], A), asthma events rate [events/day, B], and salbutamol use rate (puffs/day, C). FEV₁: forced expiratory volume in 1 s * significant correlation for P < 0.05.

Table 2

Summary of most common adverse events (n and %).

	Doxofylline 400 mg (n = 309)
Subjects with one or more adverse events	169 (54.69)
Body as a whole disorders	
Headache	44 (14.24)
Abdominal pain	13 (4.21)
Asthenia	12 (3.88)
Chest pain	8 (2.59)
Infection	7 (2.27)
Digestive disorders	
Nausea	45 (14.56)
Dyspepsia	31 (10.03)
Diarrhoea	8 (2.59)
Anorexia	7 (2.27)
Nervous system disorders	
Insomnia	33 (10.68)
Nervousness	20 (6.47)
Dizziness	11 (3.56)
Respiratory system disorders	
Asthma	23 (7.44)
Pharyngitis	10 (3.24)

with the current World Health Organization (WHO), International Clinical Trials Registry Platform (ICTRP), and the International Committee of Medical Journal Editors (ICMJE) guidelines. The inclusion of these studies in a public registry has been performed not only to support transparency in clinical trials reporting [37], but also in agreement with the fundamental scientific and ethical responsibility that all research on humans used to advance knowledge should not remain invisible or abandoned, thus rejecting the culture of data secrecy [38].

Another interesting finding is that we have found a significant linear correlation between the serum levels of doxofylline and the reduction in the use of salbutamol as rescue medication, but not with respect to FEV₁. This evidence suggests that doxofylline administered at 400 mg induces ceiling bronchorelaxant effect regardless of the achievable serum concentrations ranging from ≈ 11 µg to ≈ 15 µg. On the other hand, it seems that greater serum concentrations permit to reduce the use of as needed salbutamol, supporting the in vitro finding that doxofylline binds to β₂-AR and activates this receptor itself. Therefore, the LESDA study is the first clinical trial that provides the indirect confirmation that doxofylline indeed took effects for relaxation of ASM by interacting with β₂-AR [39]. This suggests that doxofylline may provide sparing effects not only with respect to corticosteroids as recently demonstrated in both an allergic and a non-allergic model of lung inflammation [4], but also to β₂-AR agonists, thus opening new horizons in the potential synergistic interaction between doxofylline, corticosteroids and β₂-AR agonists combined as dual or triple therapy for the

treatment of chronic obstructive respiratory disorders [40–43]. However, since the use of concomitant medications was not included in the endpoints of LESDA study, except for salbutamol administered as rescue medication, this trial cannot provide the clinical evidence of the impact of doxofylline on the reduction in the use of any other drug such as ICSs. Certainly, this is an interesting topic worthy of further investigation, and effectively a crossover randomized clinical trial aimed to assess the efficacy of doxofylline as a sparing treatment for ICSs in asthmatic children has been recently registered in the [ClinicalTrials.gov](https://clinicaltrials.gov) repository database (NCT03879590).

The AEs recorded during the LESDA study were not serious and they were generally mild or moderate in severity, with an interesting ≈ 14% reduction in the frequency of headache when compared with data from DOROTHEO 1 and 2 studies [10]. As for other drugs that inhibit different PDE isoforms [44], this difference suggests that the long-term administration of doxofylline may induce tolerance with respect to some specific AEs, namely headache that was one of the most frequent AEs detected after few weeks of treatment with doxofylline [10].

Concluding, this study provides the evidence that doxofylline is effective and well tolerated when administered chronically to patients with poorly controlled asthma.

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CRediT authorship contribution statement

Luigino Calzetta: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Maria Gabriella Matera:** Data curation, Writing - original draft, Writing - review & editing. **Marc F. Goldstein:** Conceptualization, Methodology, Investigation, Data curation. **William R. Fairweather:** Conceptualization, Methodology, Investigation, Data curation. **William W. Howard:** Conceptualization, Methodology, Investigation, Data curation. **Mario Cazzola:** Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Paola Rogliani:** Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pupt.2019.101883>.

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