ORIGINAL ARTICLE

Calcium Channel Blocker Reduces Airway Remodeling in Severe Asthma

A Proof-of-Concept Study

Pierre-Olivier Girodet[1,2,3], Gaël Dournes[1,2,3], Matthieu Thumerel[1,2,3], Hugues Begueret[3], Pierre Dos Santos[1,2,3], Annaig Ozier[1,2,3], Isabelle Dupin[1,2], Thomas Trian[1,2], Michel Montaudon[1,2,3], François Laurent[1,2,3], Roger Marthan[1,2,3], and Patrick Berger[1,2,3]

[1]Université de Bordeaux, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, Département de Pharmacologie, CIC1401, Bordeaux, France; [2]INSERM, Centre de Recherche Cardio-thoracique de Bordeaux, U1045, CIC1401, Bordeaux, France; and [3]CHU de Bordeaux, CIC1401, Service d'Exploration Fonctionnelle Respiratoire, Service d'Imagerie Diagnostique et Thérapeutique, Service d'Anatomopathologie, Service de Chirurgie Thoracique, Service de Cardiologie, Pessac, France

Abstract

Rationale: Severe asthma is a major public health issue throughout the world. Increased bronchial smooth muscle (BSM) mass, a characteristic feature of airway remodeling in severe asthma, is associated with resistance to high-intensity treatment and poor prognosis. *In vitro*, the Ca^{2+}-channel blocker gallopamil decreased the proliferation of BSM cells from patients with severe asthma.

Objectives: We conducted a double-blind, randomized, placebo-controlled study to evaluate the effect of gallopamil on airway remodeling in patients with severe asthma.

Methods: Subjects received either gallopamil (n = 16) or placebo (n = 15) for 1 year and were monitored for an additional 3-month period. Airway remodeling was analyzed at baseline and after treatment phase using both fiberoptic bronchoscopy and computed tomography scan. The primary end point was the BSM area. Secondary end points included normalized BSM thickness and frequency of asthma exacerbations.

Measurements and Main Results: BSM area was reduced in the gallopamil group (baseline vs. end of treatment) but was unchanged in the placebo group. Between-group differences in BSM area were not significantly different in gallopamil versus placebo groups. By contrast, between-group differences in normalized BSM thickness were significantly different between the two groups. The mean number of exacerbations per month was not different during the treatment phase in gallopamil versus placebo group but was significantly lower in patients previously treated with gallopamil during the follow-up period. There were no differences between the groups with respect to overall side effects.

Conclusions: Gallopamil treatment for 12 months reduces BSM remodeling and prevents the occurrence of asthma exacerbations.

Clinical trial registered with www.clinicaltrials.gov (NCT 00896428).

Keywords: asthma; remodeling; smooth muscle; mitochondria; exacerbation

Severe asthma is a chronic airway disease that generates major consequences on morbidity, quality of life, and economic burden (1, 2). One of its main prognostic factors is the occurrence of bronchial smooth muscle (BSM) remodeling, corresponding to an increased BSM mass (3). BSM remodeling results from a complex process, mainly involving an increased proliferation of BSM

(Received in original form October 20, 2014; accepted in final form January 27, 2015)

Supported by Bordeaux University Hospital, a grant from the French Ministry of Health (PHRC Remodel'asthme, 2008, CHUBX 2008/09), and a grant from the French National Institute of Health and Medical Research (Recherche clinique translationnelle INSERM/INCa/DHOS A08032GS).

Author Contributions: Conception and design, P.-O.G. and P.B. Analysis and interpretation, P.-O.G., G.D., M.T., H.B., P.D.S., A.O., I.D., T.T., M.M., F.L., R.M., and P.B. Drafting the manuscript for important intellectual content, P.-O.G., R.M., and P.B. Revising the manuscript for important intellectual content, P.-O.G., G.D., M.T., H.B., P.D.S., A.O., I.D., T.T., M.M., F.L., R.M., and P.B. Final approval of the manuscript, P.-O.G., G.D., M.T., H.B., P.D.S., A.O., I.D., T.T., M.M., F.L., R.M., and P.B.

Correspondence and requests for reprints should be addressed to Patrick Berger, M.D., Ph.D., Centre de Recherche Cardio-thoracique de Bordeaux, INSERM, U1045, Université de Bordeaux, 146 rue Léo Saignat. 33076 Bordeaux Cedex, France. E-mail: patrick.berger@u-bordeaux.fr

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 191, Iss 8, pp 876–883, Apr 15, 2015

Originally Published in Press as DOI: 10.1164/rccm.201410-1874OC on January 29, 2015
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Severe asthma is a major cause of chronic morbidity, mortality, and health care costs. Increased bronchial smooth muscle (BSM) mass, a key feature of airway remodeling, is associated with the risk of asthma exacerbations and lung function decrease. The Ca^{2+}-channel blocker gallopamil decreased the proliferation of BSM cells from patients with severe asthma *in vitro*.

What This Study Adds to the Field: This translational clinical trial is the first to assess the long-term (12 months) effect of a Ca^{2+}-channel blocker on airway remodeling in patients with severe asthma. Compared with placebo, gallopamil added to conventional antiinflammatory treatment significantly reduced normalized BSM thickness. This potential add-on treatment may provide a new, safe, and beneficial approach to BSM remodeling consequences in severe asthma.

cells, which has been demonstrated *ex vivo* (4) and *in vitro* (5). To date, active pharmaceutical compounds able to decrease BSM size within the whole bronchial tree are still lacking (6). In particular, corticosteroids, which represent the gold standard of asthma therapy, remain unable to decrease the proliferation of asthmatic BSM cells (7).

Using BSM cells obtained from people with severe asthma, we have previously demonstrated that such an increased proliferation is triggered by extracellular calcium (Ca^{2+}) influx, which subsequently activates mitochondrial biogenesis (8). The Ca^{2+}-channel blocker gallopamil, also known as D600 or methoxyverapamil, abolished this Ca^{2+} rise and inhibited transcription factors expression involved in mitochondrial biogenesis, resulting in a reduction in mitochondrial mass and BSM cell proliferation. However, the clinical effect of gallopamil on BSM remodeling has not yet been evaluated in clinical trials of sufficient duration and power.

The objectives were to study the efficacy and safety of adding oral gallopamil for 12 months, as compared with placebo, to a treatment regimen of inhaled corticosteroids and long-acting β-agonists in patients with severe asthma, in a randomized, double-blind, placebo-controlled, parallel-group trial. We evaluated the effect of gallopamil on BSM mass, wall thickness assessed by computed tomography (CT), exacerbation frequency, and other end points during a 15-month period in patients with severe asthma. Indeed, we hypothesized that gallopamil could decrease BSM size. Because BSM represents a large proportion of the bronchial wall thickness in people with severe asthma, this reduced BSM size could also decrease wall thickness.

Methods

Participants

Subjects aged more than 18 years were eligible for enrollment if they had a clinical diagnosis of asthma including characteristic symptoms (i.e., wheezing and breathlessness) (9) and bronchial hyperresponsiveness confirmed either by a significant improvement by greater than 15% in the FEV_1 10 minutes after the inhalation of 200 μg of salbutamol, or a provocative concentration of methacholine required to lower the FEV_1 by 20% of less than 4 mg/ml according to the American Thoracic Society criteria (10). Main inclusion criterion was a diagnosis of severe asthma according to the American Thoracic Society criteria (11). Main exclusion criteria were current smoking or former smoking with more than 10 pack-years or less than 3 years after quitting and recent asthma exacerbation (<6 wk). Additional criteria for exclusion were those related to contraindications to gallopamil or bronchoscopy. Details of inclusion and exclusion criteria and permitted and excluded concomitant medications are provided in the online supplement.

All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki. All subjects provided written informed consent. The ethics committee (Comité de Protection des Personnes) of South West area (France) approved the research protocol on June 24, 2009.

Study Design and Treatment

The study was a single-center, randomized, double-blind, placebo-controlled, parallel-group clinical trial conducted from December 2009 through February 2014. Bertin Pharma (Artigues-Près-Bordeaux, France) provides contract pharmaceutical services including gallopamil importation (Abbott, Wiesbaden, Germany), placebo manufacturing, and stability testing according to Europe and US Food and Drug Administration Good Manufacturing Practices standards.

The statistician of the Methodology and Data management Centre established the randomization list before the start of the study. Eligible patients were initially enrolled by clinical investigators (P.-O.G., A.O., and P.B.) and were randomly assigned in a 1:1 ratio to receive either 100 mg of oral gallopamil hydrochloride twice daily or a matching placebo. The allocation sequence was created using SAS version 9.0 (SAS Institute, Inc., Cary, NC) with a block size of four. A document describing the randomization procedure was kept, confidentially, by the Methodology and Data management Centre. Gallopamil and placebo tablets were identical in color, shape, and taste. All patients and investigators were masked to treatment allocation. To ensure full masking, an independent nurse and a qualified cardiologist were involved in the trial. At each patient visit, investigators were not aware of heart rate, blood pressure, and electrocardiogram results.

A 3-month run-in period was designed to optimize treatments of both asthma and comorbidities. There were then 15 visits: a visit at the start of the trial (inclusion visit 3 mo before randomization [M−3]), a visit at randomization (baseline, Month 0 [M0]), 12 visits every month during the 12-month treatment period (M1–M12), and one additional visit at the end of the 3-month follow-up at the 15th month (M15). During the 12-month treatment period, a prevention of exacerbation plan was followed (*see* Table E1 in the online supplement). Such an action plan has been shown to decrease bronchial inflammation (12). Indeed, it was our hypothesis that mitochondrial biogenesis could be activated not only by gallopamil-dependent calcium pathway but also by inflammatory pathways (13). Each randomized patient underwent two fiberoptic bronchoscopies and two CT scans at M0 and M12. Airway remodeling was assessed by two complementary methods: first, on bronchial specimens, the BSM area was automatically

assessed on six to eight sections per biopsy and three biopsies per subject using optic microscopy, α-smooth muscle staining by immunohistochemistry, and Quancoul software (Quant'Image, Bordeaux, France), this latter being able to automatically recognize any prespecified color, as described previously (14, 15); and, second, on CT images, the wall thickness was assessed using a Laplacian of Gaussian algorithm (16) and a three-dimensional analysis software (17).

Outcome Measures

The primary end point was the BSM area assessed as the percentage of BSM surface on the whole bronchial sections surface. Secondary outcomes were bronchial wall thickness, normalized BSM thickness, frequency of asthma exacerbations, Asthma Control Questionnaire (ACQ), short-acting β-agonists use, Asthma Quality of Life Questionnaire (AQLQ), FEV_1, fractional exhaled nitric oxide (F_{ENO}), lung hyperinflation (VI950) or air trapping (VE850, difference or ratio between inspiratory and expiratory mean lung density), epithelial area, subepithelial membrane thickness, and lamina propria thickness (*see* METHODS in the online supplement for a complete description). Normalized BSM thickness was calculated and expressed in micrometers. The BSM thickness was obtained by multiplying the percentage BSM area by the bronchial wall thickness measured by CT, both from the fourth generation (*see* Figure E1).

Statistical Analysis

We determined that with 16 patients in each group, the study would have a power of 80% to detect an absolute between-group difference of 13.45 ± 12.70 percentage points in the percent decrease in normalized BSM size, taking into account a 10% drop out rate.

According to Bland-Altman analysis (18, 19), the reproducibility and the repeatability of BSM area measurements were evaluated after log transformation of data. Comparisons between groups (i.e., placebo vs. gallopamil) were performed by Fisher exact test or chi-square for comparison of proportions, unpaired *t* tests for comparison of parametric variables, and Mann-Whitney *U* tests for comparison of nonparametric variables. Comparisons within group after and before treatment were performed by means of paired *t* tests or Wilcoxon rank tests for parametric or nonparametric variables, respectively. Pearson or Spearman correlation matrix was built for parametric or nonparametric variables, respectively. Exacerbation frequency was calculated and compared between the study groups with the use of a negative binomial model and verified with the Mann-Whitney *U* test. Values are presented as mean ± SD in the tables and mean ± SEM in the figures. The statistical analysis was performed with NCSS 2001 software (NCSS Statistical Software, Kaysville, UT).
A *P* value less than 0.05 was considered statistically significant.

Results

Enrollment and Baseline Characteristics

Figure 1 shows the numbers of patients who were screened, enrolled, and randomly assigned to a study group and who completed the study. A total of 31 of the 36 patients who were included started treatment. Fifteen patients were randomly assigned to receive placebo. Only one patient from each group was withdrawn (i.e., at M10 in the gallopamil group and

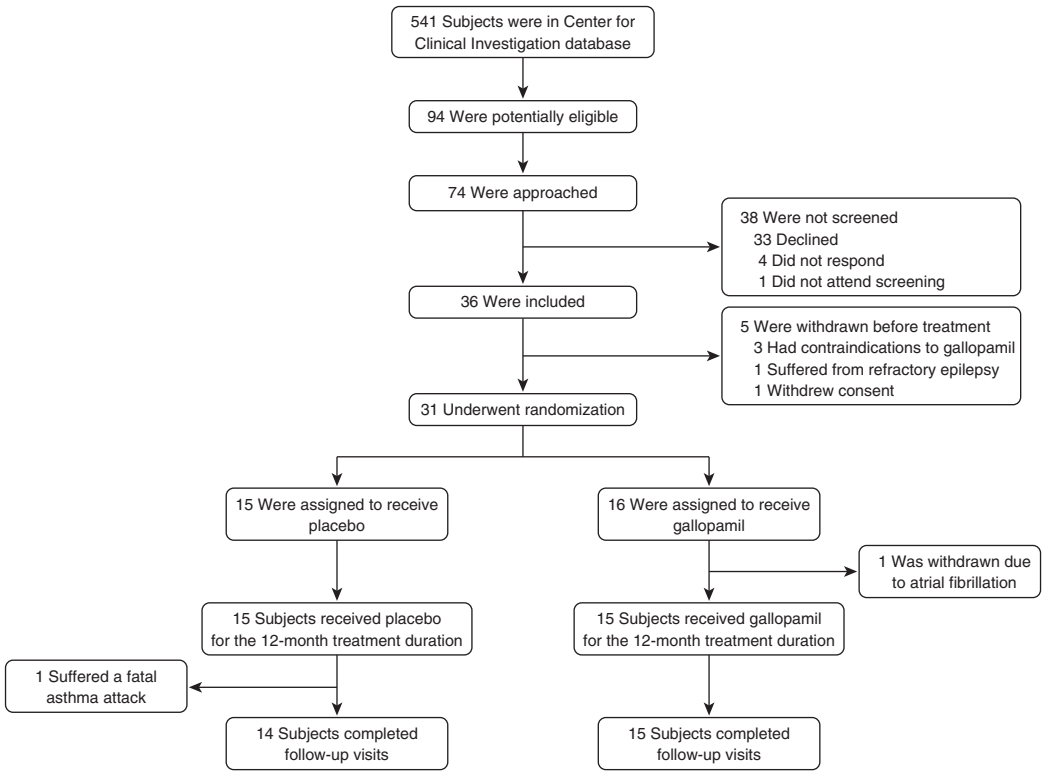

Figure 1. Flowchart of study design. Number of patients who were screened, enrolled, and assigned to a study group and who completed the study.

after M12 in the placebo group). Subjects in the two groups were well matched with respect to baseline characteristics at M0 (Table 1; *see* also the study protocol in Figure E2). Moreover, there was no difference between the placebo and gallopamil groups in terms of intensity of inhaled or oral corticosteroid during the first 3-month run-in period and during the 12-month treatment phase of the study. The trial ended when the last patient performed the last visit.

Efficacy

BSM remodeling. BSM area was assessed at M0 and M12 (*see* Figure E3). The median treatment period was 352 days in the gallopamil group and 345 days in the placebo group ($P = 0.36$). Intraobserver and interobservers reproducibility of BSM area was assessed (*see* Table E2). Between and within-biopsies variability of BSM area were similar in gallopamil versus placebo groups at both M0 and M12 (*see* Table E3).

First, the comparison between the within group differences in BSM area between M12 and M0 was not significantly different in gallopamil ($-3.4 \pm 1.3\%$; 95% confidence interval [CI], -6.1 to -0.8) versus placebo groups ($-2.8 \pm 1.6\%$; 95% CI, -6.1 to 0.5) (Figure 2B, $P = 0.75$). However, BSM area assessed at M12 was reduced as compared with that assessed at baseline within gallopamil group only (Figure 2A). This effect of gallopamil was restricted to the BSM layer because it did not alter the epithelial area, the subepithelial membrane thickness, or the lamina propria thickness assessed at M12 as compared with those assessed at baseline (*see* Figure E4).

To define a more robust parameter of BSM mass, we assessed the normalized BSM thickness, as a secondary outcome. Such a normalized BSM thickness (presented in micrometers) was calculated by multiplying normalized BSM area (in percentage) by the bronchial wall thickness (in millimeters) measured by CT at the same bronchial generation (i.e., the fourth generation). The median normalized BSM thickness significantly decreased from 402 μm at baseline to 330 μm at the end of the treatment period in the gallopamil group ($P = 0.01$), whereas that of the placebo group remained unchanged from 351 to 363 μm ($P = 0.56$) (Figure 2C). Moreover, the within-group difference in normalized BSM thickness between M12 and M0 (i.e., the delta of the placebo [5.5 ± 28.5 μm; 95% CI, -55.5 to 66.6] vs. the delta of the gallopamil [-70.4 ± 22.9 μm; 95% CI, -119.5 to -21.3]) was significantly different ($P = 0.03$) (Figure 2D). The mean percentage of variation in the normalized BSM thickness decreased by 13.7% within the gallopamil group, whereas it remained nearly stable (i.e., increased by only 2.2%) within the placebo group.

We also assessed the number of mitochondria per BSM cell surface (*see* Figure E5A), but neither gallopamil nor placebo altered it (*see* Figure E5B). However, the number of mitochondria was significantly and positively correlated with BSM area and normalized BSM thickness at both M0 and M12 (*see* Figure E6).

Wall thickness. Wall thickness of bronchi from multiple generations (second, third, and fourth) was assessed at both M0 and M12 using CT and dedicated software (*see* Figure E7). The significant decrease in normalized BSM thickness induced by gallopamil was not related to a significant decrease in wall thickness assessed at the fourth bronchial generation (*see* Table E4). By contrast, the mean wall thickness, assessed from the second to the fourth generation, was significantly decreased at M12 as compared with that assessed at baseline in the gallopamil group ($P = 0.04$) (Figure 3A), whereas that of the placebo group remained unchanged. However, the comparison between the within-group differences (i.e., the delta of the placebo vs. the delta of the gallopamil effect on mean wall thickness between M12 and M0) was not significantly different in gallopamil (-0.08 ± 0.04 mm; 95% CI, -0.16 to 0.0004) versus placebo groups (0.01 ± 0.02 mm; 95% CI, -0.04 to 0.05; $P = 0.06$) (Figure 3B). Similar results

Table 1. Patient Characteristics

	Placebo	Gallopamil	*P* Value
N	15	16	
Age, yr	58.7 ± 11.9	55.1 ± 13.7	0.45
Sex, M/F	2/13	4/12	0.65
Score on Juniper Asthma Control Questionnaire	2.3 ± 1.5	2.3 ± 1.5	0.98
Age at onset of symptoms			
Early/late	6/9	6/10	1.00
Year	17.1 ± 13.3	17.0 ± 14.9	0.74
Asthma duration, yr	41.6 ± 19.4	38.1 ± 16.9	0.60
High eosinophil status, Y/N	9/6	11/5	0.72
Blood eosinophil count, $\times 10^{-9}$/L	0.22 ± 0.20	0.22 ± 0.23	0.77
F_{ENO}, ppb	28.8 ± 26.7	22.2 ± 17.6	0.71
Prebronchodilator FEV_1, % of pred. value	69.8 ± 23.1	78.0 ± 23.5	0.34
FEV_1:FVC ratio, %	62.5 ± 11.4	69.4 ± 8.6	0.06
Obesity, Y/N	4/11	4/12	0.27
Body-mass index, kg/m^2	27.6 ± 5.0	28.9 ± 7.0	0.92
Smoking status			
Current smoker, Y/N	0/15	0/16	1.00
Ex-smoker, Y/N	2/13	4/12	0.65
Pack-years, no.	0.7 ± 1.9	1.2 ± 2.6	0.47
Frequent exacerbations >2/yr, Y/N	6/9	11/5	0.16
Severe exacerbations per subject in previous year, no.	2.9 ± 3.0	3.6 ± 2.9	0.28
Treatment			
Dose of ICS, beclomethasone equivalent, μg/day	3,000 ± 1,732	2,744 ± 1,029	0.97
Use of LABAs, Y/N	15/0	16/0	1.00
Regular use of oral prednisolone, Y/N	5/10	1/15	0.08
Use of montelukast, Y/N	7/8	8/8	1.00
Use of omalizumab,Y/N	6/9	5/11	0.72

Definition of abbreviations: F_{ENO} = the fraction of nitric oxide in exhaled air; ICS = inhaled corticosteroids; LABAs = long-acting β-agonists.
Plus-minus values are means ± SD.
P values were calculated with the use of a two-sided independent *t* test for variables with a parametric distribution, Fisher exact test for comparison of proportions, and the Mann-Whitney *U* test for comparison of nonparametric variables.

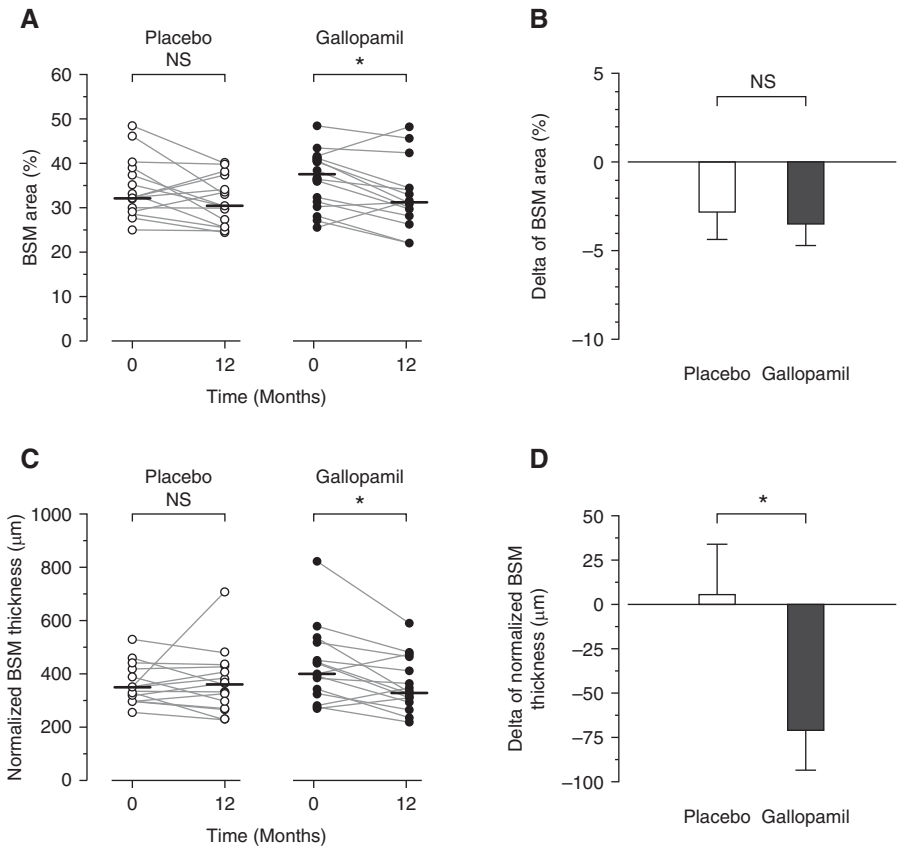

Figure 2. Effect of gallopamil on bronchial smooth muscle (BSM) remodeling. The area of BSM layer was assessed using Quancoul software on bronchial biopsy sections at a magnification of ×100. This BSM area was expressed as percentages of BSM area on the whole bronchial section area (*A* and *B*). Computed tomography–normalized BSM thickness is BSM area multiplied by bronchial thickness of fourth generation assessed by three-dimensional computed tomography (*C* and *D*). The within-group (*A* and *C*) and the between-group (*B* and *D*) differences are shown. The delta of the placebo and the delta of the gallopamil effect are the differences between post-treatment values (Month 12) and pretreatment values (Month 0) and are expressed as mean ± SEM (*B* and *D*). *Open circles* and *open bars* = placebo (n = 15); *solid circles* and *solid bars* = gallopamil (n = 15). Medians are represented as *horizontal lines* (*A* and *C*). NS = nonstatistically significant. *$P < 0.05$ using paired Wilcoxon test (*A* and *C*) and Mann-Whitney test (*B* and *D*).

were obtained when the mean wall thickness was normalized to bronchial diameter ($P = 0.03$) (Figure 3C). Again, the comparison between the within-group differences were not significantly different in gallopamil (−1.2 ± 0.5%; 95% CI, −2.2 to −0.1) versus placebo groups (0.1 ± 0.6%; 95% CI, −1.1 to 1.3; $P = 0.10$) (Figure 3D). Furthermore, other CT parameters (*see* Methods in the online supplement) reflecting lung hyperinflation (VI950) or air trapping (VE850, difference or ratio between inspiratory and expiratory mean lung density) were unchanged (*see* Figure E8).

Frequency of exacerbations. During the treatment period between M0 and M12, which also included the prevention of exacerbation plan based on monitoring of eosinophilic airway inflammation, a total of 51 exacerbations occurred in the group of patients who were assigned to receive gallopamil and 60 in the group assigned to receive placebo (Figure 4A). During this period, the mean number of exacerbations per subject and per month was 0.28 in the gallopamil group, as compared with 0.33 in the placebo group (relative risk, 0.81; 95% CI, 0.47–1.42; $P = 0.46$) (Figures 4A and 4B; *see* Figure E9A). Moreover, the number of exacerbations between M0 and M12 was significantly and positively correlated with normalized BSM area assessed at M0 in the placebo group (Spearman correlation coefficient, $r = 0.55$; $P = 0.03$). By contrast, there was no correlation in the gallopamil group (Spearman correlation coefficient, $r = 0.35$; $P = 0.20$).

However, during the follow-up period between M12 and M15, which did not include the prevention of exacerbation plan, an additional five exacerbations occurred in the gallopamil group, as compared with 20 in the placebo group (Figure 4A). During this second period, the mean number of exacerbations per subject and per month was 0.10 in the gallopamil group, as compared with 0.45 in the placebo group (relative risk, 0.23; 95% CI, 0.081–0.68; $P = 0.008$) (Figures 4A and 4B). Moreover, none of the patients in the gallopamil group had more than one exacerbation during the follow-up period, as compared with 26.7% in the placebo group ($P = 0.03$) (Figure 4C). As for the treatment period, the number of exacerbations between M12 and M15 was still significantly correlated with normalized BSM area assessed at M12 in the placebo group (Spearman correlation coefficient, $r = 0.75$; $P = 0.001$) and there was no correlation in the gallopamil group too (Spearman correlation coefficient, $r = 0.23$; $P = 0.41$).

Other outcomes. During the treatment period, gallopamil did not alter any other outcomes, such as ACQ, short-acting β-agonists use, AQLQ, FEV_1, and F_{ENO} (*see* Figures E9B–E9F).

Safety

During the treatment period, the frequency of adverse events was not significantly different between patients assigned to receive gallopamil or placebo (*see* Table E5). In particular, gallopamil did not alter cardiovascular outcomes, such as heart rate, blood pressure, and PR interval (*see* Figure E10).

Discussion

The present study demonstrates that an orally administrated pharmacologic compound (i.e., gallopamil) significantly decreases the secondary outcome BSM thickness in a placebo- controlled, double-blind, 12-month trial performed in patients with severe asthma. However, although the primary outcome (i.e., BSM area) was significantly decreased in the gallopamil group, there was no difference in the delta of BSM area between placebo and gallopamil groups. This decrease in BSM remodeling resulted in a significant reduction in

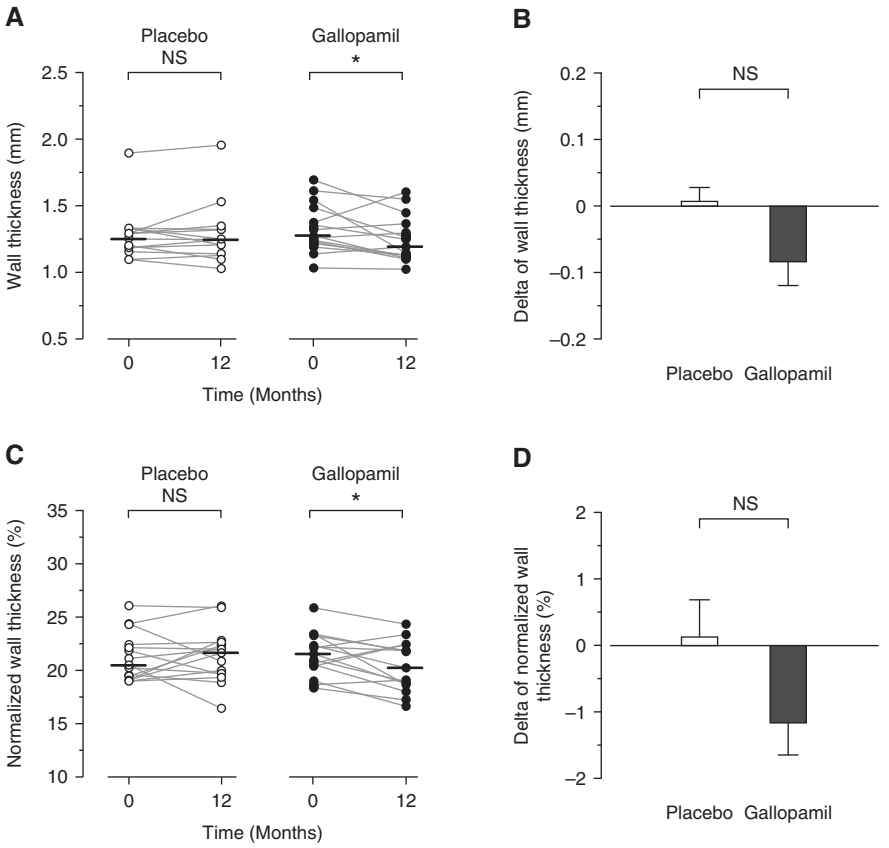

Figure 3. Effect of gallopamil on wall thickness (WT). Bronchial WT and normalized wall thickness (WT%) were assessed by using three-dimensional computed tomography on bronchial cross-sections of right lung from the second to the fourth bronchial generation according to the Boyden classification. WT% is the ratio of WT to bronchial diameter. The within-group (*A* and *C*) and the between-group (*B* and *D*) differences are shown. The delta of the placebo and the delta of the gallopamil effect are the differences between post-treatment values (Month 12) and pretreatment values (Month 0) and are expressed as mean ± SEM (*B* and *D*). *Open circles* and *open bars* = placebo (n = 15); *solid circles* and *solid bars* = gallopamil (n = 15). Medians are represented as *horizontal lines* (*A* and *C*). NS = nonstatistically significant. *$P < 0.05$ using paired Wilcoxon test (*A* and *C*) and Mann-Whitney test (*B* and *D*).

bronchial wall thickness and was associated with a reduction in subsequent asthma exacerbations during the follow-up. To date, pharmacologic compounds able to target BSM remodeling in asthma are still lacking (6). Bronchial thermoplasty, which is supposed to reduce BSM mass, significantly decreases the exacerbation rate (20). Because we previously demonstrated the antiproliferative effect of gallopamil *in vitro* using BSM cells obtained from patients with severe asthma (8), we thus undertook the present clinical trial in patients with severe asthma.

The study was powered to assess BSM size difference from baseline to post-treatment values by means of paired comparisons of BSM size determined invasively using biopsies obtained per bronchoscopy. We paid special attention to analyze BSM size in three different biopsies from each patient and in six to eight sections for each biopsy to limit the effect of within-patient variability (21). Moreover, we also attempted to evidence the significant reduction of BSM mass, in a noninvasive manner, by assessing a concomitant reduction of bronchial wall thickness measured by CT. To this end, we used customized three-dimensional software enabling bronchial tree skeletonization, orthogonal reconstruction of the main bronchial axis, and measurement of cross-sectional wall thickness, as previously described (17). One may suggest, however, that wall area would have been more appropriate than wall thickness. However, wall area is more complex to analyze because it can be influenced by bronchial perimeter and/or wall thickness. Indeed, bronchial perimeter may be altered by bronchodilation (22). Nevertheless, it is unlikely that gallopamil could have induced BSM relaxation because neither FEV_1 nor lumen area assessed by CT (data not shown) were altered.

We have used an innovative method to normalize BSM size by assessing BSM thickness, combining BSM area from various bronchial biopsies and sections, all originating from the fourth bronchial generation (i.e., the sum of BSM areas divided by the sum of whole section areas) and normalized this percentage with the bronchial thickness assessed by CT at the same fourth bronchial generation. We thus have measured a homogeneous parameter, which is normalized BSM thickness at the fourth bronchial generation, and showed that the mean percentage of variation in the normalized BSM thickness selectively decreased within the gallopamil group. Whereas gallopamil did significantly decrease bronchial wall thickness, it should be kept in mind that bronchial wall thickness in asthma can also be influenced by bronchial inflammation in addition to remodeling (23). However, in the present study there was no difference between gallopamil and placebo groups in bronchial inflammation assessed by F_{ENO} and eosinophilic blood count. In addition, there was a significant and positive correlation between BSM mass and wall thickness thus suggesting that the decrease in wall thickness was mainly caused by the decrease in BSM mass. Moreover, we also assessed other components of the bronchial remodeling (i.e., epithelium, subepithelial membrane, and lamina propria thickness) and did not find any effect induced by gallopamil, further supporting that its effect was restricted to the BSM layer. However, BSM size in severe asthma depends on complex mechanisms involving increased BSM cell proliferation, BSM cell migration, fibrocytes recruitment, extracellular matrix protein deposition, and potentially decreased BSM cell apoptosis (24).

During the 12-month treatment phase, we monitored eosinophilic airway inflammation to prevent asthma exacerbations. Indeed, it was our hypothesis that reversing BSM remodeling required the control of inflammatory pathways, which could induce BSM cell proliferation

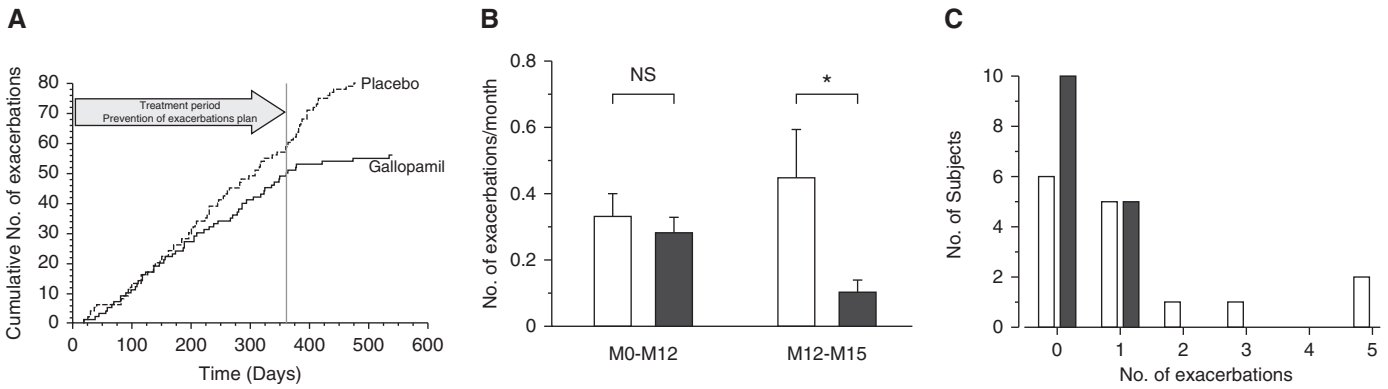

Figure 4. Effect of gallopamil on asthma exacerbations. (*A*) Cumulative number of severe exacerbations that occurred in placebo (*dashed line*) and gallopamil (*solid line*) groups. The *vertical gray line* represents the start of follow-up. (*B*) Number of exacerbations per month in placebo (*open bars*, n = 15 and n = 14) and gallopamil (*solid bars*, n = 15 and n = 15) groups over investigational product administration (M0–M12) and follow-up (M12–M15) periods, respectively. Values are means ± SEM. NS = nonstatistically significant. $^*P < 0.05$ using Mann-Whitney test. (*C*) Distribution of the number of exacerbations among subjects in placebo (*open bars*) and gallopamil (*solid bars*) groups during the follow-up period of the study.

(15), in addition to the inhibition of the calcium-induced transduction pathway previously demonstrated *in vitro* (8). For this purpose, we adapted for patients with severe asthma (*see* Table E1) the treatment hierarchy previously developed by Green and coworkers (12). As a consequence, the significant reduction in the exacerbation frequency occurred subsequently to the gallopamil treatment phase, not only when the BSM mass has been significantly decreased but also when the prevention of exacerbation plan has been withdrawn. Likewise, in the anti–IL-13 dupilumab clinical trial, the efficacy of dupilumab on the exacerbation frequency appeared only when the corticosteroid treatment was withdrawn (25).

By contrast, in the present study, the significant reduction in exacerbation frequency only occurred when gallopamil was withdrawn, which deserves further comments. It should be noted that the present study was not powered to show a significant reduction in asthma exacerbation frequency and that a minimal number of exacerbations before enrollment was not required as an inclusion criteria. Moreover, it can be speculated that a treatment phase of 12 months was not enough to decrease exacerbation frequency. In addition, the dose of gallopamil could have been insufficient to demonstrate a significant effect on exacerbations. In the present study, however, gallopamil was unable to significantly alter any clinical parameter.

In terms of safety, gallopamil did not induce noticeable adverse events. For instance, there was no significant reduction in heart rate or blood pressure. Accordingly, no effects on blood pressure or heart rate were reported in healthy volunteers (26, 27).

In conclusion, this pioneer clinical trial provides a proof-of-concept that gallopamil, which is able to block BSM cell proliferation *in vitro* (8), is also able to significantly reduce normalized BSM thickness *in vivo* after 12-month treatment in patients with severe asthma. Whereas the other secondary outcomes (i.e., ACQ, AQLQ, FEV_1 exacerbations) were not significantly altered by gallopamil during the treatment phase, there was a significant decrease in asthma exacerbations during the follow-up. Further studies using larger cohorts of patients are needed to confirm these results and to investigate the preventive and/or the curative effect of the Ca^{2+}-channel blocker gallopamil on asthma exacerbations. However, this proof-of-concept study validates that, besides the invasive bronchial thermoplasty procedure, a pharmacologic strategy can reverse BSM remodeling leading to beneficial clinical outcomes. Moreover, because reduction in bronchial wall thickness has also been noninvasively confirmed using CT, alternative calcium inhibitors or combinations of gallopamil with new antiinflammatory compounds may now be undertaken to optimize such pharmacologic strategy. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank the study participants, Regis Lassalle for statistical support, the staffs of both pathology and surgery departments, Elise Maurat, Virginie Niel, and Thomas Royo-Lazaro for technical assistance.

References

1. Currie GP, Douglas JG, Heaney LG. Difficult to treat asthma in adults. *BMJ* 2009;338:b494.
2. Martinez FD, Vercelli D. Asthma. *Lancet* 2013;382:1360–1372.
3. Kaminska M, Foley S, Maghni K, Storness-Bliss C, Coxson H, Ghezzo H, Lemiere C, Olivenstein R, Ernst P, Hamid Q, *et al*. Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction. *J Allergy Clin Immunol* 2009;124:45–51, e41–44.
4. Ramos-Barbón D, Fraga-Iriso R, Brienza NS, Montero-Martínez C, Verea-Hernando H, Olivenstein R, Lemiere C, Ernst P, Hamid QA, Martin JG. T cells localize with proliferating smooth muscle alpha-actin+ cell compartments in asthma. *Am J Respir Crit Care Med* 2010;182:317–324.
5. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, Burgess JK, Black JL. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474–477.
6. Girodet PO, Ozier A, Bara I, Tunon de Lara JM, Marthan R, Berger P. Airway remodeling in asthma: new mechanisms and potential for pharmacological intervention. *Pharmacol Ther* 2011;130:325–337.

7. Roth M, Johnson PR, Borger P, Bihl MP, Rüdiger JJ, King GG, Ge Q, Hostettler K, Burgess JK, Black JL, *et al*. Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N Engl J Med* 2004; 351:560–574.
8. Trian T, Benard G, Begueret H, Rossignol R, Girodet PO, Ghosh D, Ousova O, Vernejoux JM, Marthan R, Tunon-de-Lara JM, *et al*. Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma. *J Exp Med* 2007;204:3173–3181.
9. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2012 [accessed 2015 Jan 23]. Available from: http://www.ginasthma.org
10. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, *et al*. Guidelines for methacholine and exercise challenge testing-1999. *Am J Respir Crit Care Med* 2000;161:309–329.
11. American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2000;162:2341–2351.
12. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715–1721.
13. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C, Sciorati C, Bracale R, Valerio A, Francolini M, Moncada S, *et al*. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* 2003;299:896–899.
14. Berger P, Lavallée J, Rouiller R, Laurent F, Marthan R, Tunon-de-Lara JM. Assessment of bronchial inflammation using an automated cell recognition system based on colour analysis. *Eur Respir J* 1999;14:1394–1402.
15. Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, Thumerel M, Ousova O, Kolbeck R, Coyle AJ, *et al*. Role of YKL-40 in bronchial smooth muscle remodeling in asthma. *Am J Respir Crit Care Med* 2012;185:715–722.
16. Berger P, Perot V, Desbarats P, Tunon-de-Lara JM, Marthan R, Laurent F. Airway wall thickness in cigarette smokers: quantitative thin-section CT assessment. *Radiology* 2005;235:1055–1064.
17. Montaudon M, Berger P, de Dietrich G, Braquelaire A, Marthan R, Tunon-de-Lara JM, Laurent F. Assessment of airways with three-dimensional quantitative thin-section CT: in vitro and in vivo validation. *Radiology* 2007;242:563–572.
18. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.
19. Bland JM, Altman DG. Measurement error and correlation coefficients. *BMJ* 1996;313:41–42.
20. Cox G, Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, Olivenstein R, Pavord ID, McCormack D, Chaudhuri R, *et al*.; AIR Trial Study Group. Asthma control during the year after bronchial thermoplasty. *N Engl J Med* 2007;356:1327–1337.
21. Jones RL, Elliot JG, James AL. Estimating airway smooth muscle cell volume and number in airway sections. Sources of variability. *Am J Respir Cell Mol Biol* 2014;50:246–252.
22. Dournes G, Berger P, Laurent F. Airway compliance studied by lumen area changes alone cannot discriminate between collapsibility and elasticity. *Am J Respir Crit Care Med* 2013;187:107–108.
23. Montaudon M, Lederlin M, Reich S, Begueret H, Tunon-de-Lara JM, Marthan R, Berger P, Laurent F. Bronchial measurements in patients with asthma: comparison of quantitative thin-section CT findings with those in healthy subjects and correlation with pathologic findings. *Radiology* 2009;253:844–853.
24. Bara I, Ozier A, Tunon de Lara JM, Marthan R, Berger P. Pathophysiology of bronchial smooth muscle remodelling in asthma. *Eur Respir J* 2010;36:1174–1184.
25. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, Wang L, Kirkesseli S, Rocklin R, Bock B, *et al*. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013;368:2455–2466.
26. Gross AS, Eichelbaum M, Mörike K, Mikus G. Pharmacokinetics and pharmacodynamics of R- and S-gallopamil during multiple dosing. *Br J Clin Pharmacol* 2000;49:132–138.
27. Schweizer MW, Brachmann J, Kirchner U, Walter-Sack I, Dickhaus H, Metze C, Kübler W. Heart rate variability in time and frequency domains: effects of gallopamil, nifedipine, and metoprolol compared with placebo. *Br Heart J* 1993;70:252–258.