

Calcium Channel Blocker Reduces Airway Remodeling in Severe Asthma

A Proof-of-Concept Study

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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 Ca²⁺-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

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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), FEV₁, fractional exhaled nitric oxide (F_{ENO}), lung hyperinflation (VE850) 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 $\pm$ SD in the tables and mean $\pm$ 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

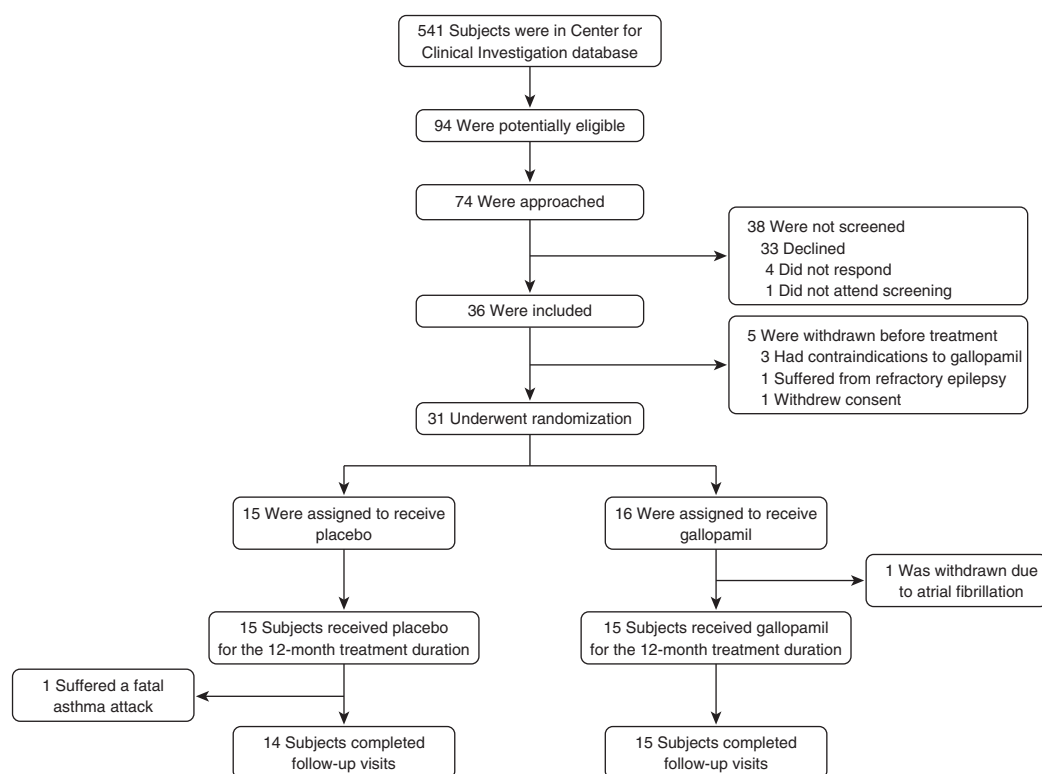


Figure 1. Flowchart of study design. Number of patients who were screened, enrolled, and assigned to a study group and who completed the study.

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 $402 \mu\text{m}$ at baseline to $330 \mu\text{m}$ 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 $363 \mu\text{m}$ ($P=0.56$) (Figure 2C). Moreover, the within-group difference in normalized BSM thickness between M12 and M0 (i.e., the delta of the placebo [$5.5 \pm 28.5 \mu\text{m}$; 95% CI, -55.5 to 66.6] vs. the delta of the gallopamil [$-70.4 \pm 22.9 \mu\text{m}$; 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 ($-0.08 \pm 0.04 \text{ mm}$; 95% CI, -0.16 to 0.0004) versus placebo groups ($0.01 \pm 0.02 \text{ mm}$; 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, $\times 10^{-9}/\text{L}$	0.22 ± 0.20	0.22 ± 0.23	0.77
FeNO , 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 $>2/\text{yr}$, 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, $\mu\text{g}/\text{day}$	$3,000 \pm 1,732$	$2,744 \pm 1,029$	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: FeNO = the fraction of nitric oxide in exhaled air; ICS = inhaled corticosteroids; LABAs = long-acting β -agonists.

Plus-minus values are means $\pm$ 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.

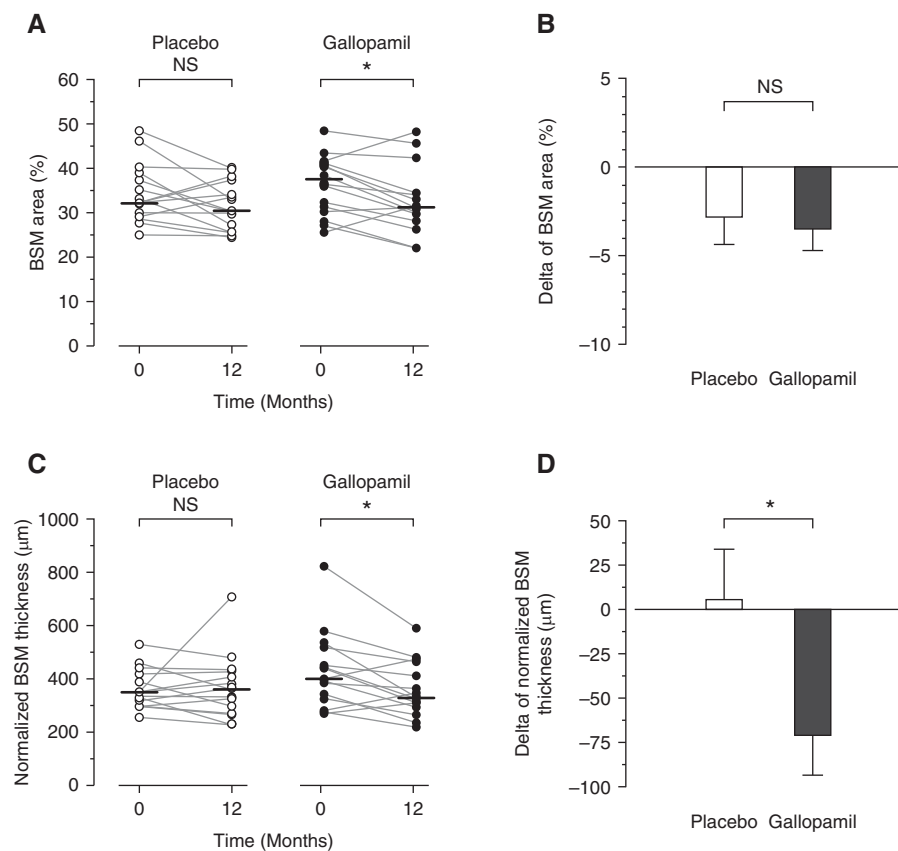


Figure 2. Effect of gallopamil on bronchial smooth muscle (BSM) remodeling. The area of BSM layer was assessed using Quancoul software on bronchial biopsy sections at a magnification of $\times 100$. This BSM area was expressed as percentages of BSM area on the whole bronchial section area (A and B). 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 $\pm$ 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 ($-1.2 \pm 0.5\%$; 95% CI, -2.2 to -0.1) versus placebo groups ($0.1 \pm 0.6\%$; 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, FEV₁, and FE_{NO} (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

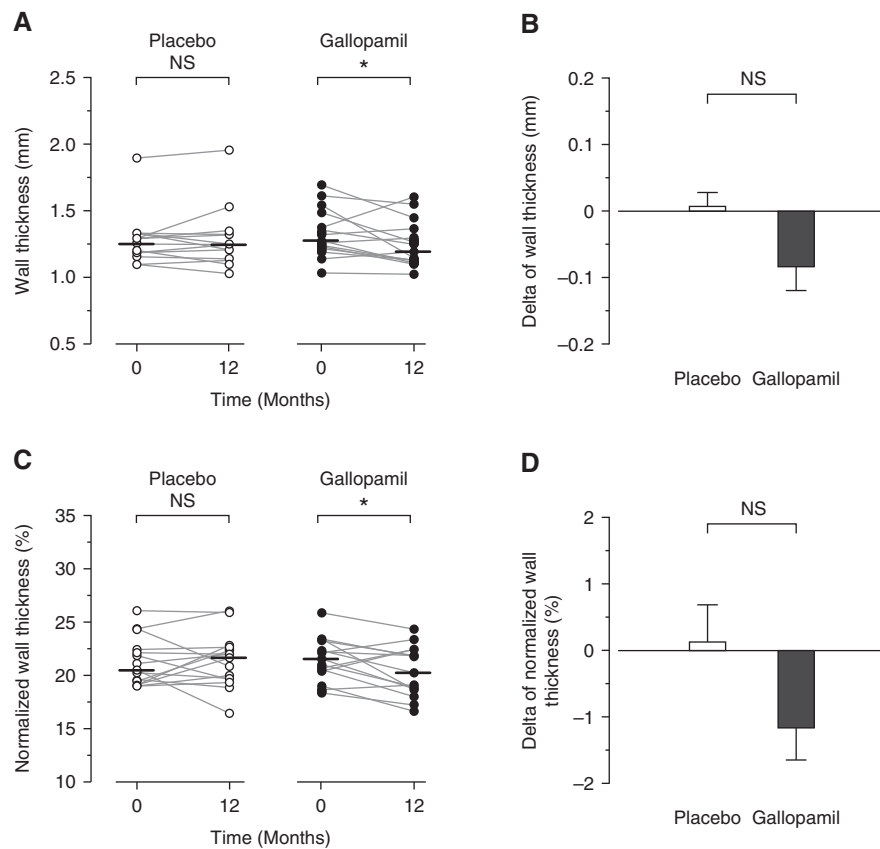


Figure 3. Effect of gallopamil on wall thickness (WT). Bronchial WT and normalized wall thickness (WT%) were assessed by using three-dimensional computed tomography on bronchial cross-sections of right lung from the second to the fourth bronchial generation according to the Boyden classification. WT% 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 $\pm$ 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 FEV₁ 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 FE_{NO} 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

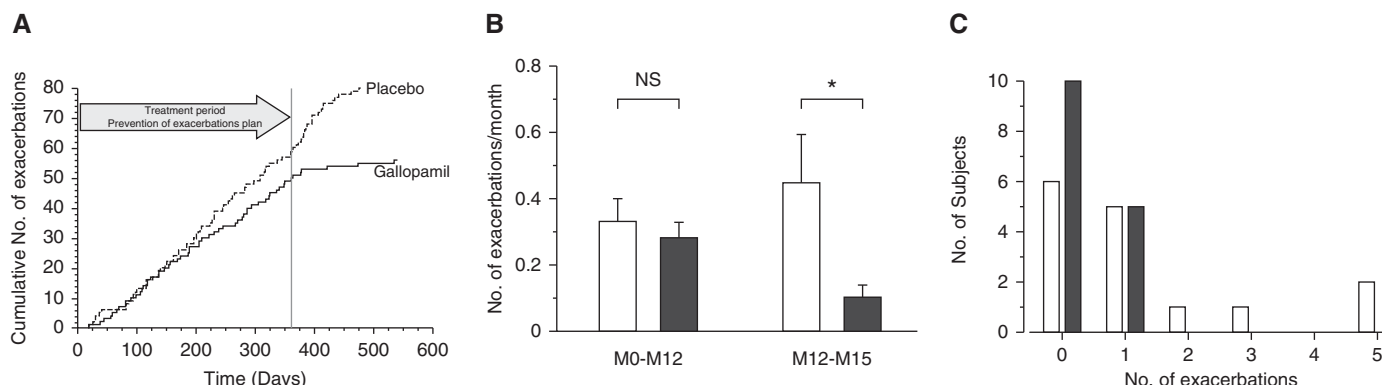


Figure 4. Effect of gallopamil on asthma exacerbations. (A) Cumulative number of severe exacerbations that occurred in placebo (dashed line) and gallopamil (solid line) groups. The vertical gray line represents the start of follow-up. (B) Number of exacerbations per month in placebo (open bars, $n = 15$ 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 $\pm$ 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, FEV₁ 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 Ca²⁺-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.

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Online Data Supplement

Calcium channel blocker reduces airway remodeling in severe asthma: a proof-of-concept study

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Methods

Subjects

Subjects aged more than 18 years were eligible for enrolment if they had a clinical diagnosis of asthma including characteristic symptoms (*i.e.*, wheezing and breathlessness) (1), as well as bronchial hyperresponsiveness confirmed either by a significant improvement by >15% in the forced expiratory volume in 1 s (FEV₁) 10 min after the inhalation of 200 µg of salbutamol, or a provocative concentration of methacholine required to lower the FEV₁ by 20% (PC₂₀) of <4 mg/ml according to the American Thoracic Society criteria (2).

Inclusion criteria were a diagnosis of severe asthma according to the American Thoracic Society criteria (3). At least one major criterion and at least two minor criteria must be fulfilled.

Major criteria:

1. Treatment with continuous or near continuous ($\geq 50\%$ of year) oral corticosteroids.
2. Requirement for treatment with high-dose inhaled corticosteroids ($> 1260 \mu\text{g/d}$ beclomethasone dipropionate or equivalent).

Minor criteria:

1. Requirement for daily treatment with a controller medication in addition to inhaled corticosteroids, *e.g.*, long-acting beta2-agonist, theophylline, or leukotriene antagonist.
2. Asthma symptoms requiring short-acting beta2-agonist use on a daily or near daily basis.
3. Persistent airway obstruction (FEV₁ < 80% predicted; diurnal PEF variability > 20%).
4. One or more urgent care visits for asthma per year.
5. Three or more oral steroid “bursts” per year.
6. Prompt deterioration with $\leq 25\%$ reduction in oral or inhaled corticosteroid dose.

7. Near fatal asthma event in the past.

Exclusion criteria were current smoking or former smoking with more than 10 pack-years or less than 3 years after quitting, recent asthma exacerbation (less than 6 weeks), chronic viral infections (HBV, HCV, HIV), aspergillosis, pregnant or nursing (lactating) women, women of child-bearing potential, prisoners, protected adults, no affiliation to the French Social Security System, patients who have received any investigational drugs within five half-lives prior to enrolment, compensation for clinical trials participation that exceed €3,800 within the previous year.

Additional exclusion criteria were those related to gallopamil treatment (*i.e.*, severe conduction disorders (2nd or 3rd degree atrioventricular block and sick-sinus syndrome, except in patients with a pacemaker), atrial fibrillation/flutter, Wolff-Parkinson-White syndrome, uncontrolled congestive heart failure, cardiogenic shock, acute coronary syndrome, hypotension (systolic blood pressure <90 mmHg), bradycardia (heart rate <50 beats/min), intraventricular conduction delay, hepatopathy, porphyria, excluded concomitant treatment and those related to bronchoscopy (*i.e.*, coagulation disorders, unstable cardiovascular conditions, a fasting state of less than 6 hours).

Permitted concomitant medications were treatment of asthma comorbidities including that of rhinosinusitis (nasal corticosteroids, anti-histaminic), gastroesophageal reflux disease (proton pump inhibitors) and *mycoplasma pneumoniae* infection (antibiotic). Excluded concomitant medications were beta-blocker, digoxin, other calcium channel blockers, other antiarrhythmic drugs, ivabradine, triazolam and dantrolene.

All subjects provided written informed consent to participate to the study, after the nature of the procedure had been fully explained. All clinical data were collected in the clinical

investigation center (CIC 1401) from the University hospital of Bordeaux (CHU). The study protocol was approved by the local research ethics committee and the French National Agency for Medicines and Health Products Safety. The study has been registered under the N° NCT00896428 at ClinicalTrials.gov (*i.e.*, “Remodel’asthme” study).

Design of the study

The study was a single-center, randomized, double-blind, placebo-controlled, parallel-group clinical trial conducted from December 2009 through February 2014. The funding organization did not include any pharmaceutical company. The study was supported by the French Ministry of Health and the French National Institute of Health and Medical Research and sponsored by University Hospital of Bordeaux (*i.e.*, “CHU de Bordeaux”). All authors were academic and made the decision to submit the manuscript for publication and vouch for the accuracy and integrity of the contents. 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 FDA Good Manufacturing Practices standards.

The study protocol is summarized in figure E1. There were fifteen visits: a visit at the start of the trial (inclusion, M-3), a visit at randomization (baseline, M0), twelve visits every month during the 12-month treatment period (M1 to M12), and one additional visit at the end of the 3-month follow up (M15).

We designed a 3-month run-in period, to optimize treatments of both asthma and comorbidities. At the inclusion visit, we initially assessed asthma control questionnaire (ACQ) (4), asthma quality of life questionnaire (AQLQ) (5), the presence of comorbidities, the concomitant treatments, the number of exacerbations in the previous year, spirometry, Fractional exhaled NO (FeNO), induced sputum and blood eosinophils.

At both the randomization visit (M0) and at the end of the treatment period (M12), we performed ACQ, AQLQ, assessment of previous exacerbations number, concomitant treatments and adverse events, spirometry, FeNO, induced sputum, blood eosinophils, computed tomography and fiberoptic bronchoscopy.

At visits M1-M11 and M15, we performed ACQ, AQLQ, assessment of previous exacerbations number, concomitant treatments and adverse events, spirometry, FeNO, induced sputum and blood eosinophils.

In order to prevent asthma exacerbations, the anti-inflammatory treatment was reinforced by 1 step, at each visit from M-3 to M11, if FeNO > 20 ppb and/or if the percentage of induced sputum eosinophils > 3%, according to the optimization process plan (Supplemental table E1), which was adapted for severe asthmatics from that developed by Green *et al* (6). Otherwise, asthma treatment remained unchanged in the absence of exacerbation. From M12 to M15, anti-inflammatory asthma treatment was unchanged in the absence of exacerbation.

Asthma exacerbations were defined as events of worsening asthma requiring oral or parenteral corticosteroids and/or antibiotics for 5 to 10 days. During the study, asthma exacerbations were managed according to GINA recommendations (1).

Computed tomography

CT protocol

CT examinations of the chest were performed with a 16-section multidetector CT scanner (Somatom Sensation 16; Siemens Medical Solution, Forchheim, Germany) without contrast medium administration by using the following parameters: 110-kV tube voltage, 50-mAs tube current and 0.75-mm collimation. Data were acquired in the supine position under spirometric gating at full inspiration (TLC) and full expiration (RV). CT datasets were reconstructed with

both high-spatial-frequency and standard algorithm, with a 1-mm reconstruction section thickness, 1-mm reconstruction interval, 320x320-mm field-of-view, and 512x512 matrix.

CT datasets of images were performed at M0 and M12 and were available in all patients enrolled in the study. The scanner was calibrated regularly with air and a water phantom to allow reliable measurements. CT datasets of images were anonymized and analyzed in random order.

Quantitative CT analysis of proximal bronchi

Datasets of images acquired at full inspiration and reconstructed with high-spatial-frequency algorithm were transferred into a workstation and images were displayed with a parenchymal window width (1800 HU) and level (-600 HU), and analyzed in three dimensions by using dedicated and validated software (7). CT measurements of intrapulmonary airways were performed within the right lung from the second to the fourth bronchial generation including three lobar, ten segmental and twenty sub-segmental bronchi according to the Boyden Classification (8). Automatic quantification of bronchial wall area (WA) and lumen area (LA) were obtained on orthogonal bronchial cross sections by using the Laplacian-of-Gaussian algorithm (9) and 3D reconstruction (10). Briefly, Laplacian filters are second derivative filters used to find areas of rapid change (edges) on images. Since they are very sensitive to noise, it is common to smooth the image (e.g., by using a Gaussian filter) before application of the Laplacian filter, a one-step process called the Laplacian of Gaussian operation (DacLoG). We have previously validated DacLoG in a two-dimensional approach (9). Neko3D is a second dedicated software tool for analyzing bronchi in three-dimensional using a skeletonization of the bronchial tree and reconstruction in two-dimensional thin-section CT images orthogonal to the main bronchial axis of bronchi in order to measure WA and LA using DacLoG. Neko3D software enables analysis of the bronchial tree according to bronchial

generation. Reproducibility of bronchial tree skeletonization has been previously evaluated in vivo. The accuracy of measurements has been previously assessed in vitro with a phantom made with silicone tubes and an excised human lung (10). Reproducibility and reliability of WA and LA has also been determined previously (10). Bronchial wall thickness (WT), bronchial diameter (D) and normalized bronchial wall thickness (WT%) were calculated as follows:

$$WT = \{\sqrt{[(WA+LA)/\pi]} - \sqrt{(LA/\pi)}\} \text{ (expressed in mm)}$$

$$D = \{\sqrt{[(WA+LA)/\pi]}\} \times 2 \text{ (expressed in mm)}$$

$$WT\% = WT/D \text{ (expressed in \%)}$$

For each bronchus, measurements were performed at three different locations into the middle third of bronchi length and averaged. WT and WT% global values per patient were calculated after averaging the whole right lung bronchi analyzed in order to give a single mean value.

Quantitative CT analysis of lung parenchyma

Automatic quantification of lung parenchyma indices of lung hyperinflation and air trapping was performed on CT images reconstructed with standard algorithm by using whole-lung densitometry at full inspiration (TLC) and full expiration (RV) with Myrian[®] software. A threshold-based technique was used to isolate lungs from the rest of thoracic structures using CT attenuation values of -500 and -1024 HU. Densitometric indices were derived from the voxel frequency distribution histogram as follows:

1. Voxel index at a threshold of -950 HU at TLC (VI950) represents the proportion of voxels of low density and expressed as a percentage of less than a threshold of -950 HU. It indicates areas of lung hyperinflation (11).

2. Voxel index at a threshold of -850 HU at RV (VE850) represent the proportion of voxels of low density expressed as a percentage of less than a threshold of -850 HU. VE850 must represent areas of air trapping (12).
3. E/I MLD and I-E MLD are indicators of air trapping calculated respectively as the ratio and the difference of mean lung density (MLD) measured at TLC (I) and RV (E) (13, 14).

Fiberoptic bronchoscopy

Bronchial specimens from all subjects were obtained by fiberoptic bronchoscopy. The subjects received 400 µg of salbutamol with the aid of an aerosol chamber to open up the airways before the procedure. Anesthesia of the upper airways was achieved with lidocaine 5% spray. The total amount of lidocaine per subject was limited to 225 mg. For 15/31 patients (6/15 and 9/16 in the placebo and gallopamil groups respectively), an additional general anesthesia was necessary using propofol (average dose of 1.5 to 2 mg/kg). The fiberoptic bronchoscope (Pentax BF 15V, Argenteuil, France) was introduced and biopsies were taken from 6 bronchial carinae from the middle lobe and from the lower right lobe. Specimens were immediately transferred to the laboratory in sterile container containing Dulbecco's Modified Eagles Medium (DMEM, Invitrogen, Cergy Pontoise, France).

Optic microscopy and immunohistochemistry

Bronchial specimens were fixed in 10% buffered formalin and processed in standard fashion for paraffin embedding as described previously (15). Sections, 3-µm thick, were cut and stained with hematoxylin-eosin-safran (HES) or processed for immunohistochemistry. After deparaffinization and rehydration through graded alcohols, endogenous peroxidase was inhibited using a solution of 0.1% sodium azide and 0.3% hydrogen peroxide (both from Sigma-Aldrich, Saint Quentin-Fallavier, France) for 30 min, followed by 2 rinses in

phosphate buffered saline (PBS, Invitrogen). Non specific staining was blocked using 10% bovine serum albumin (BSA, Dako, Trappes, France) for 30 min. Sections were incubated for 2 h at room temperature with mouse anti-human smooth muscle actin (clone 1A4) or the unrelated mouse antibody (both from Sigma-Aldrich). After rinsing in PBS, biotinylated rabbit anti-mouse F(ab')₂ (Dako) was applied to the sections for 2 h, and followed by the streptavidin-biotin horseradish-peroxidase complex (Dako) for a further 2 h period. After rinsing in PBS, 1 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (Dako) plus 0.02% hydrogen peroxide for 6 min were used as substrate to develop a peroxide-dependent brown color reaction at room temperature. Finally, the sections were rinsed and counterstained with Mayer's hematoxylin.

The total area of BSM layer was assessed, automatically in a blinded fashion using Quancoul[®] software (Quant'image, Bordeaux, France) at a magnification of x 100, as described previously (13, 15-18). Briefly, each section was blinded and the area of BSM was automatically assessed using pre-specified ranges for the 3 parameters (i.e., optic density, hue and hue density (16) determined previously (18). Sometimes however, smooth muscle actin staining from blood vessels must be discarded manually. For each patient, 3 biopsies were collected and the BSM was analyzed in 6 to 8 sections in each biopsy. The primary outcome, which was the total BSM area, assessed in the multiple sections and biopsies, was normalized by the corresponding whole tissue area and presented as percentages of whole area. The normalized BSM thickness (secondary outcome) was calculated and expressed in μm . Such a BSM thickness was obtained by multiplying the % BSM area, by the bronchial wall thickness measured by CT, both from the 4th generation.

In addition, we also assessed other components of airway remodeling using Quancoul[®] software at a magnification of x 100 (epithelial area, lamina propria thickness) and x 400 (subepithelial membrane thickness). As for BSM area, the epithelial area, assessed in the

multiple sections and biopsies, was normalized by the corresponding whole tissue area and presented as percentages of whole area. For subepithelial membrane thickness and lamina propria thickness, a minimum of 20 measurements were performed and averaged on each section (19).

Electron microscopy

Specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon as described previously (20). For each specimen, semi-thin sections (1 μm -thick) were cut and stained with alkaline toluidine blue. We selected the first semi-thin section large enough to span from the epithelium to the muscular layer. Five ultrathin serial sections (60 nm-thick) were then cut on diamond knives. Four of these latter sections were subsequently randomly selected and placed on grids. Staining was performed with uracile acetate and lead citrate. Grids were then scanned by transmission electron microscopy (EM, Tecnai 12, Philips) and examined by a pathologist from left to right and from top to bottom to locate every whole nucleated BSM. Each ultrathin section was examined in its entirety. To evaluate the number of mitochondria, computerized photographs and measurements were performed in a blinded fashion by using Scanview software (Soft Imaging System) at a standard magnification of $\times 4200$. Mitochondrial densities were performed using ImageJ 1.42q free software.

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 (21, 22), the reproducibility of BSM area was evaluated by two authors (P.O.G. and T.T.) after log transformation of data by using (a) the Pearson correlation coefficient, (b) the lack of agreement (*i.e.*, bias estimated with the mean difference and the standard deviation of the difference), and (c) the intraclass correlation coefficient. The repeatability of BSM area was also analyzed by the same two authors as follows: (a) The measurement error was evaluated with the Spearman rank correlation coefficient between the individual subject standard deviations and their means, (b) The mean within-subject standard deviation also was evaluated.

Comparisons between groups (*i.e.* placebo *vs.* gallopamil) were performed by Fisher's exact test or Khi-2 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 before and after 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 $\pm$ SD in the tables and mean $\pm$ SEM in the figures. The statistical analysis was performed with NCSS 2001 software. A p value less than 0.05 was considered statistically significant.

This trial is registered with ClinicalTrials.gov, number NCT00896428.

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Supplemental figures legends

Figure E1. Schematic representation of normalized bronchial smooth muscle thickness calculation.

Representative images of a bronchial biopsy section (4th generation) stained with an anti-human smooth muscle actin antibody at a magnification of x40 (A, B). Bronchial smooth muscle (BSM) appears in yellow following automatic color recognition by Quancoul software (B). BSM area was expressed in % of the whole biopsy area. IHC: immunohistochemistry. Bars represent 50 μ m. Wall thickness (WT) was assessed in mm from CT scan images (C, D). C is a representative image obtained from a 4th generation bronchus after an orthogonal reconstruction to the main bronchial axis using Neko3D software. Bronchial wall area was automatically recognized using DacLoG software (D). Virtual representation of the bronchial wall (E). The normalized BSM thickness was obtained by multiplying the % BSM area by the bronchial WT, both from the 4th generation.

Figure E2. Schematic representation of the study design.

Treatments of asthma and comorbidities were optimized during a 3-month run-in period. This period was followed by a double-blind, randomized, placebo-controlled treatment period (day 1 to month 12). Computed tomography and fiberoptic bronchoscopy were performed before and after treatment period. At monthly study visits, assessments included asthma control questionnaire, asthma quality of life questionnaire, number of exacerbations, concomitant treatments and adverse events, spirometry, FeNO, induced sputum and blood eosinophils. Efficacy and safety continued to be monitored during the follow-up period (month 12 to month 15).

Figure E3. Effect of gallopamil on bronchial smooth muscle area.

Representative optic microscopic images from bronchial sections stained with HES were obtained from severe asthmatics in placebo (A, B) and gallopamil (C, D) groups at baseline (A, C) and at the end of 12-months treatment period (B, D). Smooth muscles were visualized (SM). Magnification: 400x. Bars: 10 μ m.

Figure E4. Effect of gallopamil on other components of bronchial remodeling.

The epithelial layer area (Panel A), the subepithelial membrane thickness (Panel B) and the lamina propria thickness (Panel C) were assessed using Quancoul[®] software on bronchial biopsy sections at a magnification of x100. The areas were expressed as percentages on the whole bronchial section area, the thicknesses were expressed in μ m. White circles: placebo (n=15); black circles: gallopamil (n=15). Medians are represented as horizontal lines. NS: not statistically significant using paired Wilcoxon tests.

Figure E5. Effect of gallopamil on the number of smooth muscle mitochondria.

Representative electronic microscopic images from bronchial sections were obtained from a severe asthmatic before (left) and after (right) 12 months treatment with gallopamil and observed at x4200 magnification (panel A). Some smooth muscle mitochondria were visualized (arrows). Bars: 1 μ m. The number of mitochondrial sections normalized on BSM cells surface was assessed from electronic microscopic images using ImageJ software (panel B). White circles: patients from placebo group (n=14). Black circles: patients from gallopamil group (n=13). Medians are represented as horizontal lines. NS: non statistically significant.

Figure E6. Correlation between BSM area and number of mitochondria in BSM cells from patients with severe asthma.

BSM area, CT normalized BSM thickness and numbers of mitochondria in BSM cells were assessed before starting treatment (visit M0, panels A and C, n=15 for both groups) and after 12 months of treatment (visit M12, panels B and D, n=14 for both groups). The correlation coefficient is from a Pearson (panels A and B) or a Spearman (panels C and D) correlation analysis. White circles: patients from placebo group. Black circles: patients from gallopamil group. BSM: bronchial smooth muscle; CT: computed tomography.

Figure E7. Effect of gallopamil on bronchial thickness.

Representative thin-section 3D-CT images from severe asthmatics obtained before (left) and after (right) 12 months treatment with placebo (panels A and B) and gallopamil (panels C and D). For each panel, left image corresponds to the original right upper lobe image after orthogonal reconstruction, black rectangle indicates RB1 bronchus, right upper and right lower images represent results from semi-automatic segmentation at x3 magnification. Quantification by using the Laplacian-of-Gaussian algorithm of both WT and WT% are given at the bottom right of each panel before (panels A and C) and after 12 months treatment (panels B and D).

Figure E8. Effect of gallopamil on densitometric CT indices of lung hyperinflation and air trapping.

Densitometric indices derived from voxel frequency histogram of lung parenchyma were quantified by using Myrian[®] software. There was no difference after 12 months in both patients treated with gallopamil (black circles, n=15) or placebo (white circles, n=15) regarding inspiratory index of emphysematous changes (VI950, panel A), expiratory index of

air trapping (VE850, panel B), or air trapping indices related with mean lung density variation between inspiration and expiration (E/I MLD, I-E MLD, panels C-D). Medians are represented as horizontal lines. NS: non statistically significant using paired *t* tests.

Figure E9. Effect of gallopamil on clinical, functional and inflammatory outcomes.

As compared with placebo (white circles, n=15), gallopamil (black circles, n=15) did not induce significant differences on number of exacerbations per month (panel A), Juniper Asthma Control Questionnaire (ACQ) score (panel B), Short-acting beta-agonists (SABA) use score (panel C), Asthma Quality of Life Questionnaire (AQLQ) score (panel D), forced expiratory volume in 1 second (FEV₁) before bronchodilator use (panel E) and Fractional exhaled nitric oxide (FeNO, panel F) over the 12 months treatment period. The term ppb denotes parts per billion. Values are means $\pm$ SEM.

Figure E10. Effect of gallopamil on cardiovascular outcomes.

Safety data show that heart rate (panel A), systolic and diastolic blood pressure (BP, panels B-C) and PR interval on electrocardiogram were not significantly different between asthmatics from placebo (white circles, n=15) group and gallopamil (black circles, n=15) group during the 12 months treatment period. Values are means $\pm$ SEM.

Supplemental tables

Table E1. Prevention of exacerbation plan

Step 1: High doses inhaled corticosteroids alone
- beclomethasone: $> 1260 \mu\text{g/d}$ and $\leq 2000 \mu\text{g/d}$
- or budesonide: $> 1200 \mu\text{g/d}$ and $\leq 1600 \mu\text{g/d}$
- or fluticasone: $> 880 \mu\text{g/d}$ and $\leq 1000 \mu\text{g/d}$
Step 2: High doses inhaled corticosteroids (as step 1) plus long acting beta-2 agonist (salmeterol or formoterol)
Step 3: Very high doses inhaled corticosteroids plus long acting beta-2 agonist
- beclomethasone: $> 2000 \mu\text{g/d}$
- or budesonide: $> 1600 \mu\text{g/d}$
- or fluticasone: $> 1000 \mu\text{g/d}$
Step 4: same as step 3 + anti-leukotriene (montelukast 10 mg/d)
Step 5: same as step 4 + short course of oral corticosteroids (prednisolone 30 mg/d for 15 d)
Step 6: same as step 4 + continues oral corticosteroids (prednisolone 30 mg/d)

Adapted from Green *et al* (6).

Table E2. Reproducibility and repeatability of BSM area in severe asthmatics

Reproducibility	Pearson	Mean Difference	ICC
	r values	± SD	
Intra-observer	0.999	0.003 ± 0.007	0.999
Inter-observer	0.993	0.005 ± 0.026	0.992

Repeatability	SD against mean - Spearman		Mean within subject SD
	r values	p values	
Intra-observer	0.168	0.249	0.004
Inter-observer	0.087	0.554	0.012

Values were obtained after log transformation of BSM areas. BSM areas were assessed from a random selection of 50 biopsies obtained at either M0 or M12. Each image collected from these biopsies was analyzed by one observer at 2 time points and by 2 observers. ICC: intraclass coefficient, Pearson: Pearson correlation coefficient, SD: standard deviation, Spearman: Spearman correlation coefficient. A low, non significant, Spearman correlation coefficient between the SD and the mean, corresponds to a reliable measurement according to Bland and Altman.

Table E3. Between and within-biopsies variability of BSM area in severe asthmatics

Variability	M0			M12		
	Placebo	Gallopamil	<i>P</i>	Placebo	Gallopamil	<i>P</i>
Between-biopsy	0.23	0.25	0.52	0.26	0.22	0.67
Within-biopsy	0.30	0.34	0.32	0.39	0.33	0.31

Data are coefficients of variation. M0: Month 0 (before treatment start); M12: Month 12 (end of treatment). *P* values are obtained using Mann-Whitney tests.

Table E4. Within-group comparisons of wall thickness assessed per bronchial generation on CT scan

Bronchial generation	Placebo			Gallopamil		
	M0	M12	<i>P</i>	M0	M12	<i>P</i>
2 nd	17.7 ± 2.5	17.5 ± 2.4	0.77	16.4 ± 1.1	16.4 ± 1.6	0.15
3 rd	21.9 ± 2.7	20.6 ± 1.9	0.13	21.2 ± 1.8	20.1 ± 1.9	0.02
4 th	21.4 ± 2.5	22.2 ± 3.8	0.18	22.4 ± 2.8	21.1 ± 2.9	0.11

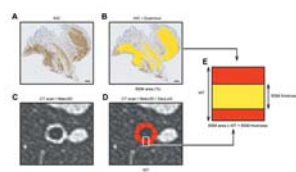
Plus-minus data are mean ± SD. M0: Month 0 (before treatment start); M12: Month 12 (end of treatment). Comparisons between M0 and M12 within each group are performed using paired t tests.

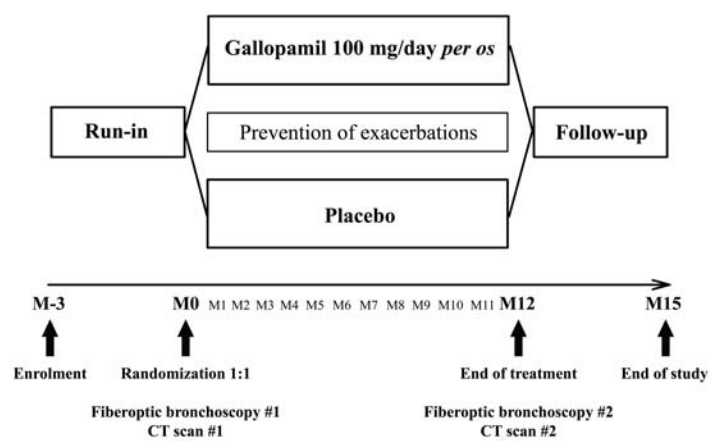
Table E5. Selected adverse events and laboratory abnormalities

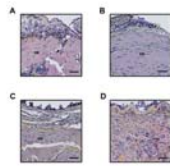
Events	Treatment group		P values
	Placebo	Gallopamil	
n	15	16	
Pulmonary event – no. (%)			
Acute bronchitis	2 (13.3)	3 (18.8)	1.00
Asthma exacerbation	13 (86.7)	15 (93.8)	0.60
Breathlessness	8 (53.3)	9 (56.3)	1.00
Bronchospasm	2 (13.3)	2 (12.5)	1.00
Thoracic pain	1 (6.7)	5 (31.3)	0.17
Cardiovascular event – no. (%)			
Atrial fibrillation	0	1 (6.3)	1.00
Bradycardia [†]	5 (33.3)	9 (56.3)	0.28
Leg edema	0	4 (25.0)	0.10
PR interval increase ^{††}	1 (6.7)	4 (25.0)	0.33
Syncope	0	1 (6.3)	1.00
Gastrointestinal or hepatic event – no. (%)			
Abnormality of liver function [#]	1 (6.7)	3 (18.8)	0.60
Acute gastroenteritis	2 (13.3)	4 (25.0)	0.65
Constipation	0	2 (12.5)	0.48
Pyrosis	0	1 (6.3)	1.00
Vomiting	0	1 (6.3)	1.00
Neurologic event – no. (%)			
Dizziness	4 (26.7)	1 (6.3)	0.17
Headache	0	4 (25.0)	0.10
Insomnia	0	1 (6.3)	1.00
Dermatologic event – no. (%)			
Erysipelas	1 (6.7)	0	0.48
Pruritus	0	2 (12.5)	0.48
Other event – no. (%)			
Diabetes	1 (6.7)	0	0.48
Otitis	1 (6.7)	0	0.48
Rhinitis	5 (33.3)	8 (50.0)	0.47
Sinusitis	2 (13.3)	4 (25.0)	0.65
Urinary tract infection	0	2 (12.5)	0.48

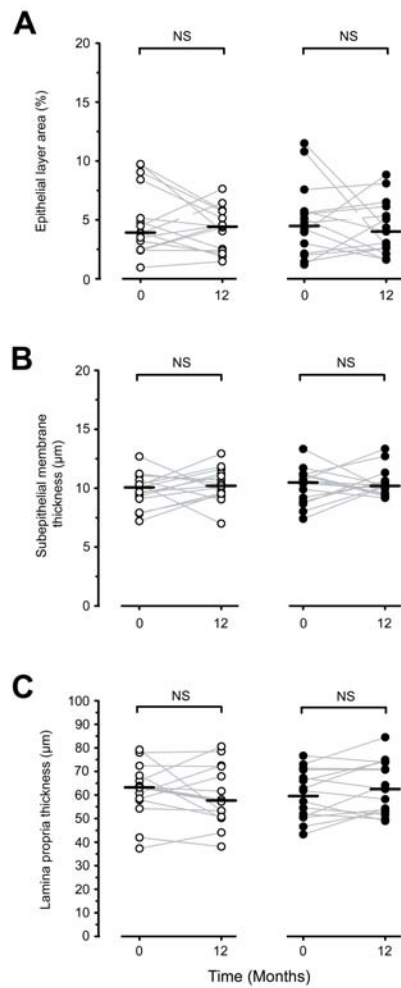
Adverse events were defined as those occurring between the first administration of a study drug and the last administration plus 2 days (half-life of gallopamil is 4 to 8h). Values are number of subjects presenting at least one adverse event. [†] Bradycardia is a heart rate of less than 60 beats per minute. ^{††}PR interval increase is PR prolongation greater than 200 ms. [#]An abnormality of liver function was defined as a level of alanine aminotransferase or aspartate aminotransferase of more than 2 times the upper limit of the normal range, an alkaline phosphatase level of more than 1.5 times the upper limit of the normal range or a total

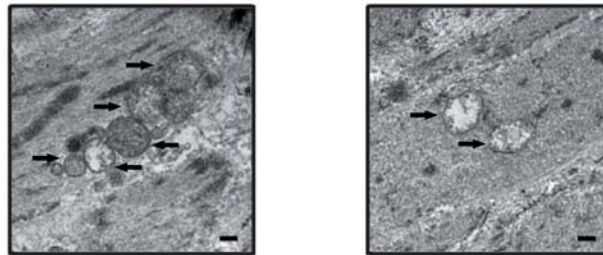
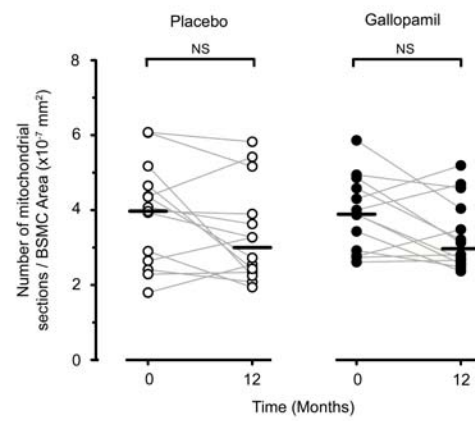
bilirubin level of 2 mg per deciliter (34 μ mol per liter) or more. P values were calculated with the use of Fisher's exact test.

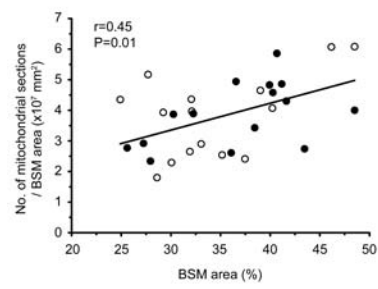
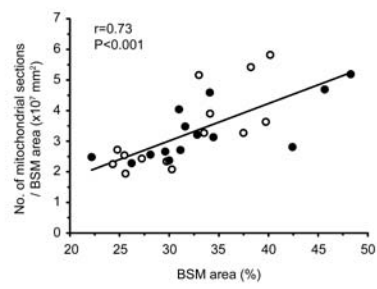
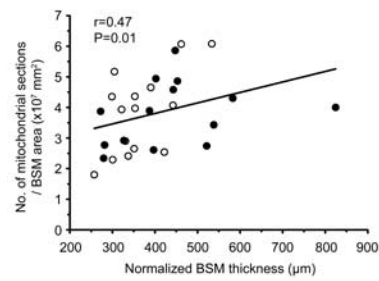
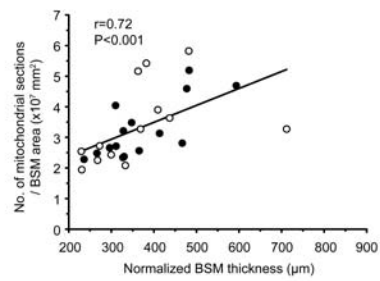


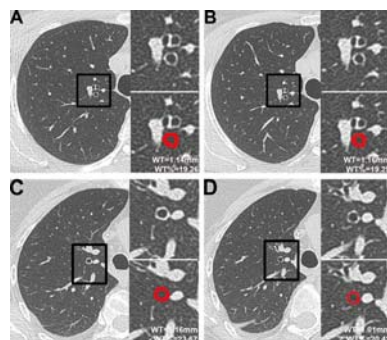


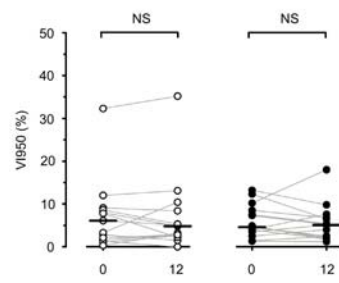
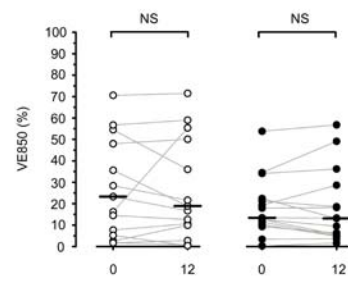
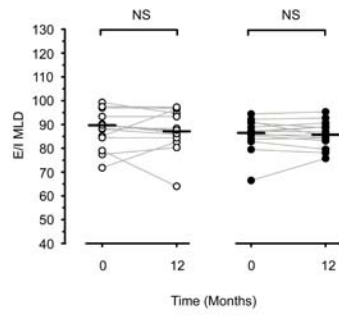
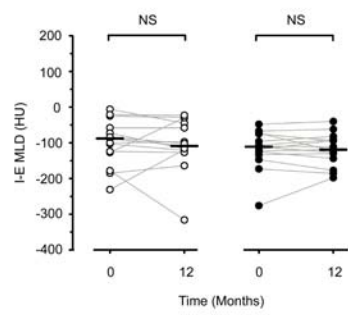


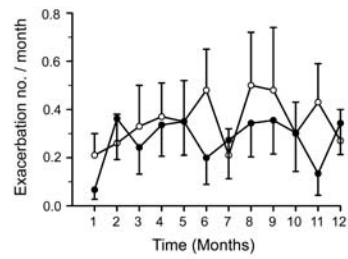
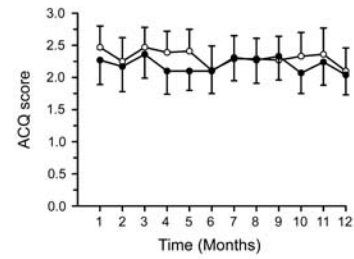
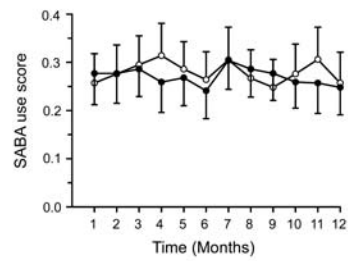
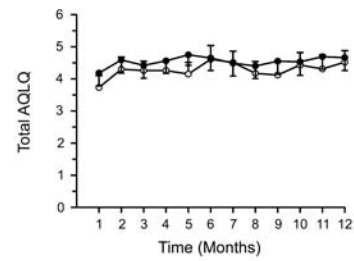
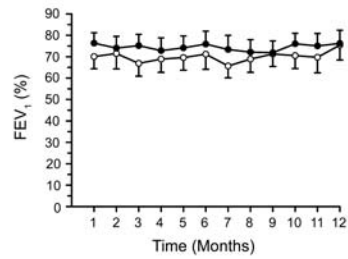
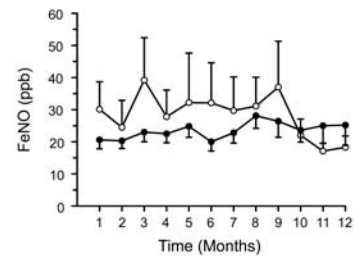


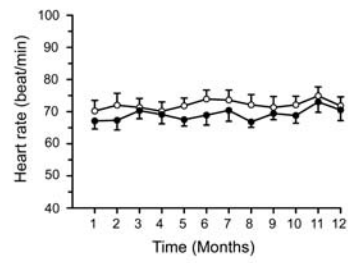
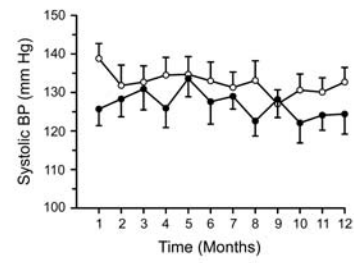
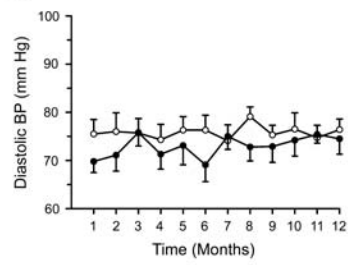
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