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Clarithromycin reduces the severity of bronchial hyperresponsiveness in patients with asthma

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ABSTRACT: A randomised double-blind placebo-controlled study was designed to evaluate the effects of a semisynthetic macrolide antibiotic, clarithromycin, on bronchial hyperresponsiveness to methacholine in patients with a diagnosis of asthma.

Adult asthma patients undergoing treatment with budesonide 400 μg *b.i.d.* and salbutamol 200 μg *p.r.n.* less than twice weekly were studied. Arm A (16 males/six females, aged 48±16 yrs) received clarithromycin 250 mg *b.i.d.* for 8 weeks, arm B (eight males/12 females, aged 42±12 yrs) clarithromycin 250 mg *t.i.d.* and arm C (six males/15 females, aged 41±16 yrs) placebo dextrose tablets. Bronchial hyperresponsiveness was quantified by measurement of the provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD_{20}).

Median (interquartile range) PD_{20} in the three groups before and after treatment with clarithromycin were: arm A: 0.3 (0.1–1) and 1.3 (0.6–2) mg; arm B: 0.4 (0.1–0.9) and 2 (2–2) mg; and arm C: 0.4 (0.1–0.9) and 0.3 (0.1–0.6) mg, respectively. Serum free cortisol levels were determined and remained unchanged from baseline in the clarithromycin-treated patients.

It is concluded that clarithromycin reduces the degree of bronchial hyperresponsiveness in patients with asthma.

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Asthma is characterised by bronchial hyperresponsiveness (BHR) to nonspecific stimuli such as histamine, methacholine, cold air and exercise. BHR is related to airway inflammation and the degree of BHR indirectly reflects the severity of the disease [1]. Systemic corticosteroids administered on a long-term basis reduce BHR, but their use has been associated with significant side-effects. Modern asthma research is orientated towards the use of inhaled corticosteroids, which also decrease BHR, or alternative drug therapies possessing less toxic immunosuppressive, immunomodulating and anti-inflammatory qualities.

Macrolides such as troleandomycin and erythromycin have been shown to be effective in the treatment of asthma [2, 3]. Their action in the steroid-dependent asthmatic patient is mediated at least in part by decreased corticosteroid metabolism. For example, troleandomycin and clarithromycin decrease methylprednisolone clearance and increase plasma methylprednisolone levels [4, 5]. However, clarithromycin does not have a significant effect on prednisolone elimination [5]. Erythromycin and especially the newer macrolides (*e.g.* roxithromycin, clarithromycin and azithromycin) exert specific anti-inflammatory effects, including inhibition of generation of reactive oxygen species by polymorphonuclear leucocytes [6, 7]. In addition, macrolide antibiotics may improve lung function due to their activity against *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, which are involved in asthma exacerbations [8–10].

As a result of the actions described above, macrolides probably reduce the severity of BHR in adults and children with asthma. Most published data on reduction of BHR refer to erythromycin, roxithromycin or azithromycin [11–13]. There are very few data on the suppression of BHR in patients with asthma following treatment with clarithromycin [14] and carefully designed controlled studies are lacking.

In the present investigation, the effects of clarithromycin on BHR in patients with bronchial asthma were evaluated. The hypothesis was that administration of clarithromycin for 8 weeks decreases the degree of BHR to methacholine.

Materials and methods

Study design

The present study was designed as a randomised double-blind placebo-controlled trial. Subjects who fulfilled the following inclusion criteria were studied: 1) age 18–70 yrs; 2) established diagnosis of bronchial asthma for $\geq$1 yr [1]; 3) treatment with budesonide 400 μg *b.i.d.* and salbutamol 200 μg *p.r.n.* less than twice weekly for $\geq$1 month prior to recruitment; and 4) a provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (FEV_1) (PD_{20}) of <2 mg.

During the study, patients continued their treatment with budesonide and salbutamol. No other medication was allowed. Exclusion criteria were as follows: 1) a history of allergic rhinitis or occupational asthma; 2) a history of smoking (past or current); 3) treatment with systemic corticosteroids or a history of upper respiratory tract infection over the 4 weeks prior to participation in the trial; 4) an FEV_1 of <50% of the

predicted value or of <1 L at baseline; 5) upper respiratory tract infection or asthma exacerbation during the study period; 6) a history of systemic diseases (*i.e.* heart attack or stroke in the previous 3 months, uncontrolled hypertension, known aortic aneurysm, epilepsy requiring drug treatment and peptic ulcer disease); 7) treatment with β-blockers; and 8) pregnancy or nursing mothers [15, 16].

After giving informed consent, each patient was randomised to one of the study groups (A–C) by a research nurse who played no further role in the study. Recruitment was discontinued when 75 patients were randomised (25 per arm). Patients and investigators were blinded with regard to the type of treatment received. The study was approved by the scientific review board of Athens Chest Hospital (Athens, Greece).

Methods

All patients were submitted to a methacholine inhalation challenge test to evaluate BHR at baseline and 24 h after completion of the trial. Patients in arm A of the study received clarithromycin 250 mg *b.i.d.* for 8 weeks. In Arm B, clarithromycin 250 mg *t.i.d* was prescribed for 8 weeks. Arm C, the control group, received placebo dextrose tablets indistinguishable from the clarithromycin tablets for 8 weeks. Bronchodilator therapy was discontinued $\geqslant$12 h before the provocation test.

The methacholine inhalation challenge method has been described in detail elsewhere [15]. In summary, ampoules of methacholine hydrochloride (Lofarma, Milan, Italy) were stored at 4°C and then kept at room temperature for 30 min before use. Aerosols of methacholine were generated using a jet nebuliser. A Dosimeter MB3 (Mefar, Bovezzo, Italy), with a valve system enabling the administration of aerosol only during inspiration, was used. A flow sensor in the expiratory port triggers a solenoid valve which exposes the nebuliser to compressed air at 138 kPa for ~0.6 s to give a calibrated output of 9.0 $\mu L \cdot puff^{-1}$.

A nose clip was used and the bolus of aerosol inhaled through the mouth over 5 s during an inspiratory capacity breath without breath-holding at total lung capacity. An aerosol of normal saline solution (diluent) was inhaled first, followed by increasing doses of methacholine (0.0156, 0.0625, 0.2590, 1.000 and 2.000 mg). The doses were administered at 5-min intervals. FEV_1 was measured 2 min after each inhalation. The test was terminated when FEV_1 had fallen by $\geqslant$20% compared to inhalation of control saline solution, or until a cumulative dose of 2 mg was reached. PD_{20} was calculated exactly [15]. For the purposes of statistical analysis, subjects who received the maximum dose (2 mg) with a fall in FEV_1 of <20% were considered to have a PD_{20} of 2 mg.

During the study, laboratory assessment of total blood count, renal function and liver function was performed biweekly. Baseline and end-of-study serum free cortisol levels were measured in a subgroup of participants.

Statistics

Descriptive statistics are presented as either median (interquartile range) or mean±SD. In each of the treatment groups (A, B or C), PD_{20} and spirometric indices before and after treatment with the study medication were compared using a Wilcoxon signed-rank test (for non-normally distributed variables) or a two-tailed paired t-test (for normally distributed variables). Changes in PD_{20} after treatment with the study medication in the 500-$mg \cdot day^{-1}$ and 750-$mg \cdot day^{-1}$ clarithromycin groups were compared using the two-independent-sample nonparametric test (Mann-Whitney test).

Results

Subject characteristics

Overall, 75 patients were recruited, 25 in each arm. However, three patients from group A were excluded from further study: one presented with an asthmatic exacerbation due to an infection and two refused to complete the 8-week treatment with the study medication. In group B, 20 patients met the eligibility criteria and five were excluded from the analysis: three presented with acute asthma exacerbation and one with a gastrointestinal disorder during the trial, whereas the final one refused to complete treatment with the study medication. In group C, 21 patients remained in the analysis as three patients presented with a severe asthma exacerbation and one did not complete the treatment and for this reason was excluded from further study.

Overall, results were analysed for 63 patients, 22 in group A, 20 in group B and 21 in group C. In groups A, B and C, Six of 22 (27%), 12 of 20 (60%) and 15 of 21 (71%) patients were female, respectively. The mean±SD age of the patients was 48±16, 42±12 and 41±16 yrs in groups A, B and C, respectively (p=NS in between-group comparisons). Mean baseline spirometric indices (FEV_1 as a percentage of the predicted value, FVC and FEV_1/FVC ratio) and median PD_{20} were similar between subjects of the three groups ($p>0.05$) (table 1).

Spirometric indices and provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second

Spirometric indices and PD_{20} before and after study medication are presented in table 1. Mean FEV_1 and FVC

Table 1. – Provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (FEV_1) (PD_{20}) and spirometric indices before and after study medication in the three groups[#]

	Group A		Group B		Group C	
	Before	After	Before	After	Before	After
Subjects n	22		20		21	
PD_{20} mg	0.3 (0.1–1)	1.3 (0.6–2)***	0.4 (0.1–0.9)	2 (2–2)***	0.4 (0.1–0.9)	0.3 (0.1–0.6)
FEV_1 % pred	85±14	85±12	85±13	88±12***	86±14	88±15
FVC % pred	96±10	96±11	92±10	92±9	87±15	89±15
FEV_1/FVC	72±14	72±11	75±5	78±5***	80±5	79±7

Data are presented as median (interquartile range) or mean±SD. FVC: forced vital capacity; % pred: per cent predicted. [#]: arm A patients received clarithromycin 250 mg *b.i.d.* for 8 weeks, arm B clarithromycin 250 mg *t.i.d* and arm C, the control group, placebo dextrose tablets indistinguishable from the clarithromycin tablets. ***: $p \leqslant 0.001$ *versus* before study medication.

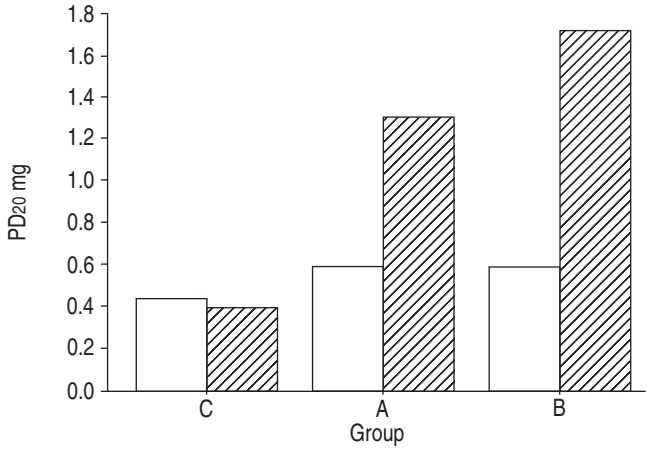

Fig. 1. – Mean provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD_{20}) at baseline (□) and after study medication (▨) in the placebo (C) and 500-mg·day^{-1} (A) and 750-mg·day^{-1} (B) clarithromycin groups. PD_{20} after treatment are higher compared to baseline in the 500-mg·day^{-1} and 750-mg·day^{-1} clarithromycin groups ($p<0.001$) but not in the placebo group.

as a percentage of the predicted value before and after treatment remained unchanged in patients of groups A and C ($p>0.05$). Compared to baseline, there was a significant increase in the median PD_{20} in the two clarithromycin-treated groups (A and B) but not in the placebo-treated group (C) (table 1; fig. 1). More specifically, median (interquartile range) PD_{20} in the three groups before and after treatment with study medication were: arm A: 0.3 (0.1–1) and 1.3 (0.6–2) mg ($p<0.001$); arm B: 0.4 (0.1–0.9) and 2 (2–2) mg ($p<0.001$); and arm C: 0.4 (0.1–0.9) and 0.3 (0.1–0.6) mg ($p>0.05$).

Individual PD_{20} data at baseline and after treatment are presented in figure 2. PD_{20} increased in 19 of the 22 subjects in group A (clarithromycin 500 mg·day^{-1}), 19 of the 20 subjects in group B (clarithromycin 750 mg·day^{-1}) and nine of the 21 subjects in group C (placebo). No subgroups of patients could be clearly identified within each treatment group. The clarithromycin effects on BHR were independent of age and sex (data not shown).

Comparison of the changes in PD_{20} (before and after study medication) between groups A and B showed a trend favouring the higher-dose regimen, which did not reach significance (Mann-Whitney test; $p=0.07$) (fig. 1). FEV_1 was higher by 3% pred in the 750-mg·day^{-1} clarithromycin group after treatment compared to baseline ($p=0.001$).

Serum free cortisol levels were measured in 40 patients. Of these, 25 belonged to groups A and B. Measurement of endogenous cortisol levels in the patients who received clarithromycin revealed no significant changes from baseline values ($p>0.05$).

Discussion

In the present investigation, BHR in patients with asthma improved significantly after completion of an 8-week clarithromycin regimen compared to placebo. The change in PD_{20} between baseline and following treatment in the 500-mg·day^{-1} clarithromycin group compared to the 750-mg·day^{-1} clarithromycin group approached but did not reach significance.

There were no significant changes in spirometric indices in any of the three groups except for FEV_1 in the 750-mg·day^{-1}-clarithromycin-treated subjects. However, a 3% change in this parameter is not clinically significant [17]. No change in mean FEV_1 as a percentage of the predicted value was noted after a 10-week trial of erythromycin administered to 23 asymptomatic subjects with asthma who were not receiving oral or inhaled corticosteroids and who had baseline FEV_1 similar to those of the patients in the present study [11]. Likewise, no change in FEV_1 or FVC was reported in a double-blind placebo-controlled study of clarithromycin administered to 17 adults with mild-to-moderate asthma [14] or in a trial of roxithromycin given to 12 asthmatic children [12]. Both medications were studied for 8 weeks. Therefore, it does not seem that clarithromycin exerts its effect on BHR in mild asthmatics *via* bronchodilation.

Several pathophysiological mechanisms could explain the clarithromycin effect on the present asthmatic subjects. The beneficial action of macrolides was initially attributed to reduced corticosteroid elimination, with troleandomycin being the characteristic example [3, 4, 18]. In the present study, no change in serum free cortisol levels was found after treatment and, therefore, it is unlikely that clarithromycin affected the metabolism of endogenous corticosteroids.

The anti-inflammatory action of macrolides may be an alternative explanation for their beneficial action on BHR. Macrolides could exert their anti-inflammatory effects *via* several antioxidant properties of their molecule, resulting in

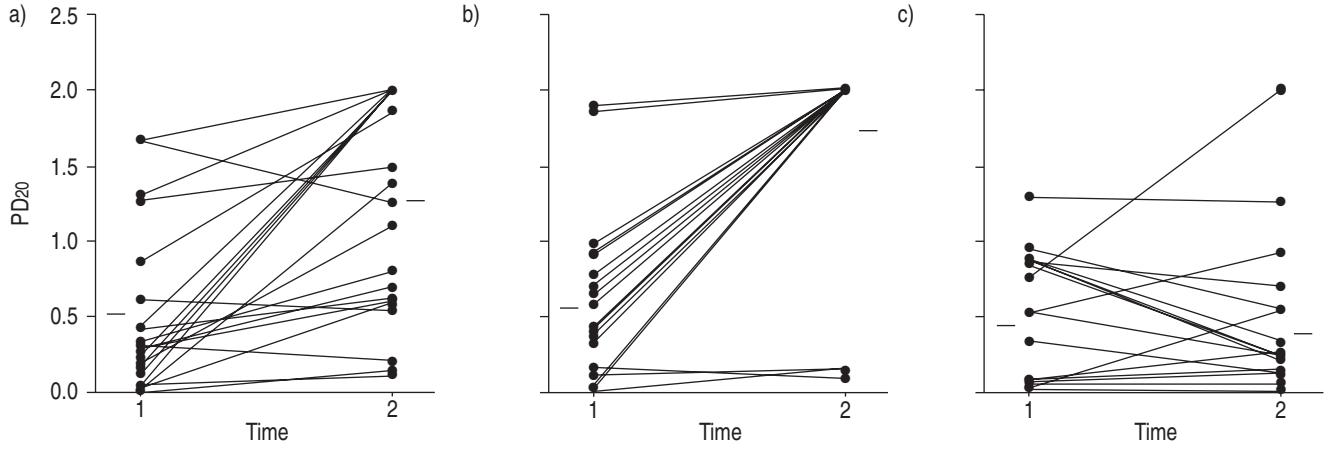

Fig. 2. – Provocative dose of methacholine causing a 20% fall in forced expiratory volume in one second (PD_{20}) at baseline (1) and after study medication (2) for individual subjects receiving a) clarithromycin 500 mg·day^{-1}, b) clarithromycin 750 mg·day^{-1} and c) placebo. Horizontal bars represent means.

inhibition of superoxide generation by polymorphonuclear leucocytes [6]. FELDMAN *et al.* [19] proposed that macrolides may have beneficial effects on airway inflammation in asthma by protecting ciliated epithelium against oxidative damage inflicted by phospholipid-sensitised phagocytes.

Interestingly, newer macrolides have inhibitory effects on cytokine secretion from leukocytes, especially interleukins-2, -3 and -4 and tumour necrosis factor-α [20], whereas erythromycin and clarithromycin exert a concentration-dependent suppressive effect on interleukin-8 release by human eosinophils from atopic donors [21]. Suppression of the interleukin-5-induced prolongation of eosinophil survival could further contribute to their effect [22]. Clarithromycin use resulted in improved symptoms as well as decreased levels of sputum eosinophils and eosinophil cationic protein in a study of asthmatic patients [14].

An effect on the neural component of airway smooth muscle contraction could explain some of the beneficial results of macrolides in asthmatic subjects. It has been suggested that their use may inhibit cholinergic neuroeffector transmission in human airway smooth muscle [23]. Furthermore, erythromycin and clarithromycin interfere with the formation of endothelin-1, a bronchoconstricting peptide which probably plays a major role in the pathogenesis of asthma [24].

Last but not least, clarithromycin could improve BHR through its antimicrobial activity since, in some individuals, infection with *C. pneumoniae* and/or other microorganisms may be associated with asthma exacerbations [8, 25, 26]. In a randomised double-blind placebo-controlled trial in asthmatic adults, treatment with clarithromycin for 6 weeks improved lung function in those who had *M. pneumoniae* or *C. pneumoniae* identified in their airways by polymerase chain reaction (PCR) [8]. Lung function did not improve in PCR-negative subjects.

The strengths of the current study include its prospective placebo-controlled double-blind nature. Additionally, by virtue of its design, it adjusts for several confounding factors, including concomitant conditions, the effect of asthma exacerbations and other medication use. Issues for further investigation are the cost-effectiveness of such an approach in the clinical setting and the duration of the effect on bronchial hyperresponsiveness, as well as the issue of the development of bacterial resistance associated with long-term antibiotic use. Nevertheless, the present investigation confirms the beneficial effect of clarithromycin use on bronchial hyperresponsiveness in patients with asthma. Clarithromycin could potentially be useful as an adjunctive therapy in the treatment of asthma.

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