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Comparative effects of a glucocorticosteroid, theophylline and the peptido-leukotriene-antagonist CGP 45715A on antigen-induced early and late phase airway response and inflammatory cell influx in sensitised guinea pigs

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Abstract

A novel model of allergic early and late-phase reaction in the airways of conscious guinea pigs was developed and the effect of established and novel antiasthmatic drugs on peak of immediate response, late phase response and associated inflammatory cell influx investigated. Guinea pigs were sensitised twice in adjuvant (50 mg/kg silica + 0.1 ml/kg Bordetella pertussis). Under cover of 10 mg/kg i.p. mepyramine guinea pigs exhibited still a pronounced immediate reaction. During a screening phase about 75% of guinea pigs demonstrated a late phase reaction of decrease of tidal volume between 4–10 h after ovalbumin inhalation. In a cross over study theophylline at 50 mg/kg p.o. (−1 h before ovalbumin) tended to attenuate not only the peak of the immediate reaction by about 69% ($P > 0.05$, $n = 12$), but inhibited the airway late phase response significantly ($P < 0.05$, 5–10 h, $n = 12$). Methylprednisolone (40 mg/kg p.o. 1 h before ovalbumin) did not inhibit the immediate response, but the late phase response. In contrast the cysteinyl-leukotriene antagonist CGP 45715A (Iralukast; 30 mg/kg p.o. 2 h before ovalbumin) neither interfered with the peak of the immediate, nor with the late phase response. When bronchoalveolar lavage by orotracheal route was performed 24 h after ovalbumin inhalation, total cell count, eosinophils, neutrophils, macrophages and lymphocytes were significantly increased in ovalbumin-controls compared to sham ($n = 5$; $P < 0.05$). Methylprednisolone reduced significantly the antigen-induced increase of total cell count and eosinophil number. Neither theophylline nor the cysteinyl-leukotriene receptor antagonist attenuated the antigen-associated cell influx. The results do not provide evidence for a major role of cysteinyl-leukotrienes in the late phase response and inflammatory cell influx in this model.

Keywords: Allergic late phase; (Guinea pig); Methylprednisolone; Theophylline; CGP 45715A; Iralukast; Cysteinyl-leukotriene; Bronchoalveolar lavage

1. Introduction

Principally the bronchoconstrictive response to a specific aerosolised antigen can result in an immediate response and in a varying proportion of asthmatic patients and animals in a second longer lasting phase of airway obstruction, the so-called late phase response. The latter lasts on average from 4 up to 24 h after antigen exposure (Larsen, 1987). The late phase response is assumed to be less sensitive to bronchodilatators (Iwama et al., 1991) and to require glucocorticosteroids for resolution.

The mechanisms which control development of the late phase response are not well understood. The same is true for the reason why only a certain percentage of asthmatics and sensitised animals develop this reaction. Sensitivity of late phase response to inhibition by glucocorticosteroids makes airway inflammation one likely contributor (Iwama et al., 1991). But more recently also long-acting β_2-adrenoceptor agonists have been described to interfere with the late phase response (Twentyman et al., 1991). Therefore, other mechanisms and mediators involved in this phenomenon have to be considered as well.

The availability of experimental late phase models in human asthmatics and animals makes animal models attractive to analyse effectiveness of clinically established and novel pharmacotherapies (bronchodilators, theo-

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phylline, corticosteroids) and to dissect mechanisms which contribute to the development of the late phase response.

The objective of the present study was to analyse and compare clinically established drugs (glucocorticosteroids, theophylline) and a new therapeutic approach of leukotriene receptor antagonism for their effect on immediate and late phase in a newly developed guinea pig model. A secondary aim was to analyse concomitantly the effect on antigen-induced inflammatory cell influx. Therefore, the effect of methylprednisolone and theophylline which are established antiasthmatic drugs was compared to the novel leukotriene-receptor antagonist CGP 45715 A (Iralukast; Von Sprecher et al., 1991).

2. Materials and methods

2.1. Study design / protocol

Male guinea pigs (Dunkin–Hartley Pirbright white, Interfauna, Tuttlingen, Germany; weight range 250–350 g at first sensitization) were sensitised twice (at a two week interval) with 20 and 10 μg ovalbumin (Sigma, D-82039 Deisenhofen, Germany), respectively, suspended in saline. Bordetella pertussis (0.1 ml/kg, 150 OU = opalescence units; Behringwerke, Marburg, Germany) and Silica (particle size 0.007 μm; 50 mg/kg; washed in ethanol and aqua dem.; Sigma, D-82039 Deisenhofen, Germany) were thoroughly mixed with the antigen-suspension and served as adjuvant. Sensitisation was performed by administration of the mixture at 2 ml per kg by i.p. route. Another group of age-matched animals were sham-sensitised with saline.

Three to four weeks after booster sensitisation animals were challenged/boosted by inhaled route with 1% ovalbumin for 2 min under CO_2-stimulation (see below). 15 min before inhalation guinea pigs received 10 mg/kg i.p.mepyramine as an antihistamine for prevention of immediate death from anaphylactic shock. Sham sensitised animals inhaled saline, respectively (Fig. 1).

Three weeks after the singular antigen inhalation sensitisation, animals were screened for presence of late phase response. After a 7 min adaptation phase in the double box plethysmograph lung function was monitored (see below) for two min (basal value). Under cover of the antihistamine mepyramine (10 mg/kg i.p. 15 min before antigen) sensitised guinea pigs were inhaled ovalbumin (1%) for 2 min and lung function monitored again at 5–15 min, and at 2, 4, 5, 6, 7, 8, 9, 10 and 24 hours after antigen. Eight sham-sensitised animals were exposed to inhalation of saline, also under pretreatment with mepyramine.

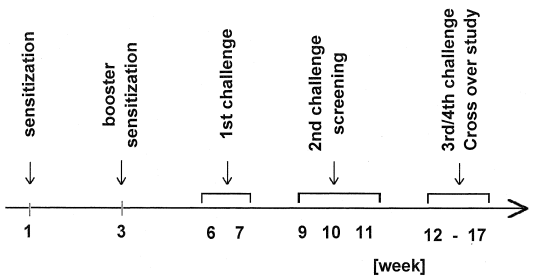

Fig. 1. Time schedule for sensitization, screening and investigation of airway late phase response in guinea pigs.

In a subsequent and parallel cross-over study the effect of following drugs was studied on the peak of immediate response (5–15 min), the late phase response and associated inflammatory cell influx: 1) methylprednisolone (Sigma, St. Louis, USA), dosed at 40 mg/kg p.o. 1 h before antigen; $n = 18$; 2) theophylline (Boehringer Ingelheim KG, D-55216 Ingelheim, Germany) 50 mg/kg p.o., 1 h before antigen, $n = 17$; and 3) the cysteinyl-leukotriene receptor antagonist CGP 45715A (Iralukast; Pharma Research, Novartis, CH-Basel, Switzerland), dosed at 30 mg/kg p.o. 2 h before antigen, $n = 15$. All test compounds were dissolved in 5% Solutol® HS 15 (BASF, Ludwigshafen, Germany) plus 0.5% methylcellulose (Tylose®) in water. Sham-sensitized- and sham-challenged animals received the same vehicle at 1 h or 2 h before inhalation of saline, respectively ($n = 10 + 10$). Each cross-over study consisted of two parts: animals which were administered during the first part test compound, received in the second part the vehicle and vice versa. The two parts of the cross over were separated by three weeks. 24 h after antigen-challenge, that was immediately after the last time point for measurement of lung function, animals were submitted to bronchoalveolar lavage via orotracheal route (see below).

2.2. Experimental set-up for measuring pulmonary function during immediate and late phase response in the conscious guinea pig

The conscious guinea pigs were placed in a cylindric double box body plethysmograph. Respiratory flow in the body plethysmograph was measured indirectly by changes in the thorax gas volume (body box) during respiration. These primary flow signals were transmitted via a differential pressure transducer (Validyne MP 45-14 (±2 cm H_2O) and an amplifier to a data acquisition/analysis system [Biowindows, software version 1.10, from Modular Instruments, Malvern, PA 19355, USA]. This system allowed recording and analysis of all raw data (respiratory flow) and derived parameters of pulmonary function (e.g., tidal volume, respiratory rate, minute volume, inspiratory time, expiratory time). For single data points, default for averaging data was set at 5 breaths. A printout of all basic data in form of a summary table and data files for further evaluation were produced.

A constant bias flow of pressurised air containing 10% CO_2 was driven at 2.5 l/min through the head box for stimulation of respiration.

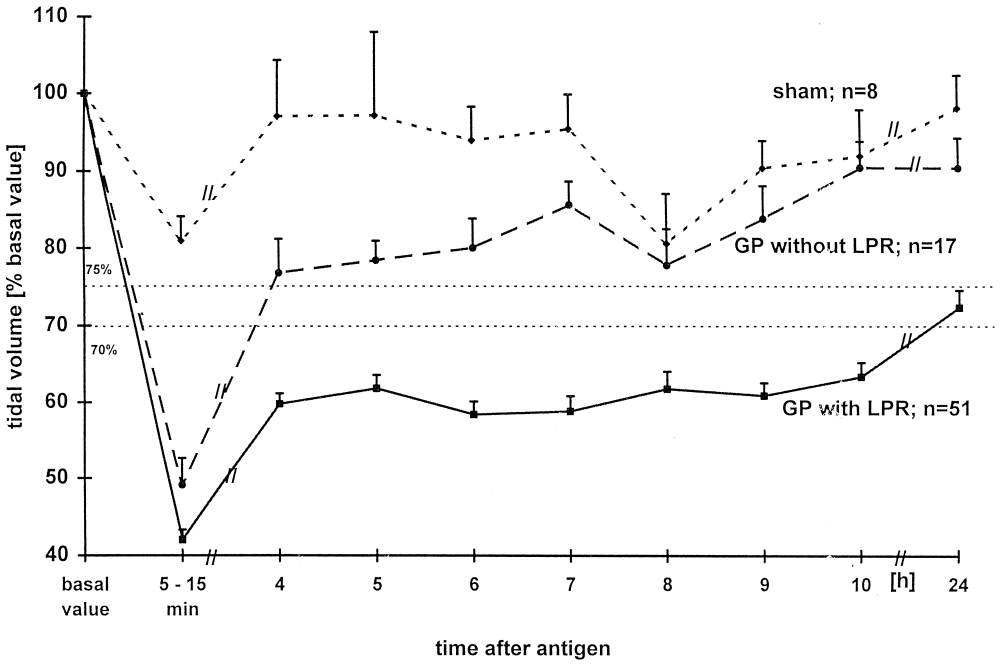

Fig. 2. Time course of tidal volume during screening for late phase responders, expressed in percent of basel in mean ± S.E.M. Criteria for presence of late phase response (LPR) in methods; guinea pigs without late phase response did not fulfill those criteria.

2.3. Performance and analysis of bronchoalveolar lavage

After the last time point for measurement of lung function (24 h) bronchoalveolar lavage (2 × 2 ml/kg saline) was performed via orotracheal route under anaesthesia with ketamin (Ketavet, Parke Davis, Freiburg; 100 mg/kg i.p.)/xylazin (Rompun, Bayer, Leverkusen; 4 mg/kg i.p.).

The absolute cell number was calculated from an aliquot counted in a Neubauer chamber. The differential cell counts (neutrophils, eosinophils, lymphocytes, macrophages, and epithelial cells) were analysed on a cytospin-preparation on the basis of at least 300 cells.

2.4. Analysis of late phase response and statistics

Late phase response during cross-over studies was present by definition if during the follow-up of lung function between 4–10 h (7 time points) tidal volume decreased

1. at least once below 70% of basal and

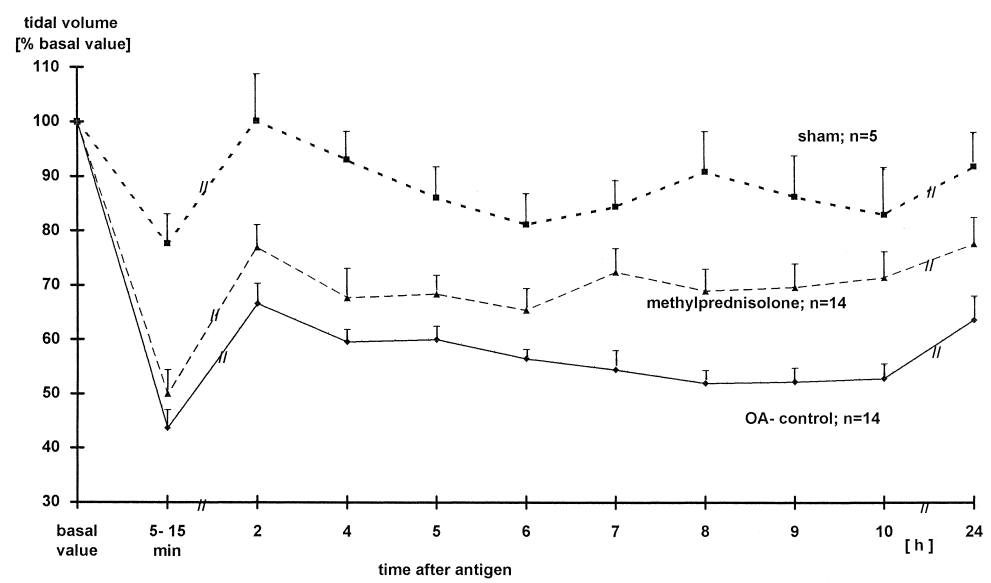

Fig. 3. Effect of oral methylprednisolone (dosed 1 h before ovalbumin-inhalation at 40 mg/kg) on tidal volume during immediate and late phase response. Time course of tidal volume, expressed in percent of basal; mean ± S.E.M. Guinea pigs which during the ovalbumin control phase did not fulfill criteria for late phase were excluded from evaluation. At time points from 6–10 h after antigen tidal volume was significantly increased in methylprednisolone treated animals (vs. ovalbumin control: $P < 0.05$).

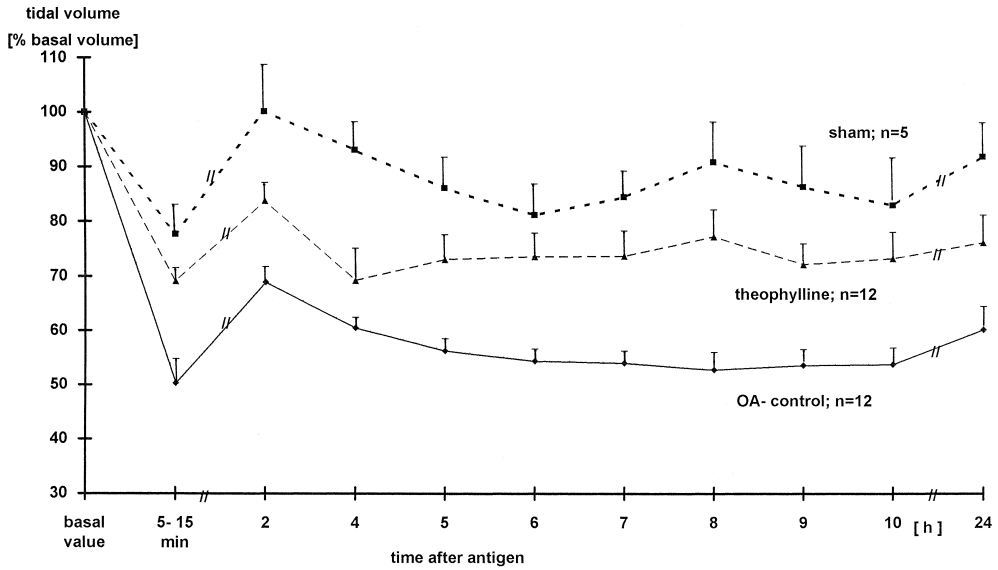

Fig. 4. Effect of oral theophylline dosed 1 h before ovalbumin-inhalation at 50 mg/kg on antigen-induced decrease of tidal volume during immediate (peak 5–15 min) and late phase response. Time course of tidal volume, expressed in percent of basal value; mean ± S.E.M. Guinea pigs which during the ovalbumin control phase did not fulfill criteria for late phase response were excluded from evaluation. At time points from 5–10 h after antigen tidal volume was significantly increased in theophlline treated animals (vs. ovalbumin control: $P < 0.05$).

2. at least 4 times (majority of time points) below 75% of basal value.

Furthermore animals under treatment during cross-over study were only evaluated if:

1. basal value of tidal volume of individual guinea pigs was higher than 3.5 ml
2. the smaller of both basal value did not deviate by more than 25% from the higher one
3. during the control/vehicle phase of cross-over animals had a late phase response according to above definition.

For statistical evaluation the mean ± S.E.M. of differences between test compound and vehicle treatment for each time point of measurement was the basis for comparing significant differences from zero by the paired Wilcoxon-test. α-adjustment was performed according to Bonferroni–Holm. For graphic presentation tidal volume was continued to be presented as mean ± S.E.M.

For testing of significant inhibition of the immediate response by the test compound (maximal decrease of tidal volume during the first 5–15 min after ovalbumin inhalation) the paired Wilcoxon test was performed without α-adjustment.

Cell counts from the bronchoalveolar lavage are presented graphically as mean ± S.E.M., significant differ-

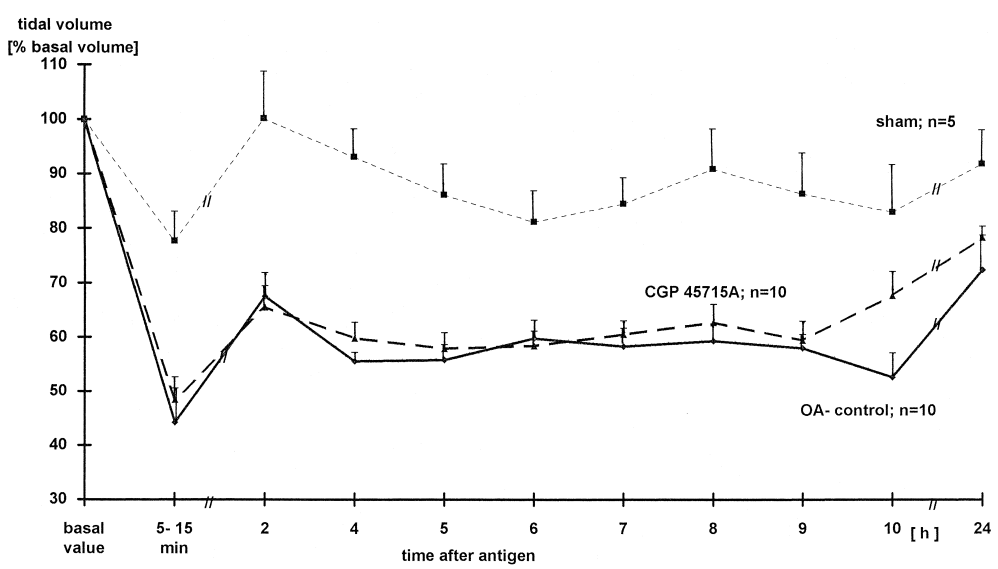

Fig. 5. Effect of oral CGP 45715A dosed 2 h before ovalbumin-inhalation at 30 mg/kg on antigen-induced decrease of tidal volume during immediate (peak 5–15 min) and late phase response. Time course of tidal volume, expressed in percent of basal value; mean ± S.E.M. Guinea pigs which during the ovalbumin control phase did not fulfill criteria for late phase response were excluded from evaluation.

ences were detected by the paired Wilcoxon test. For testing differences from sham treated animals the unpaired Wilcoxon test was calculated.

3. Results

3.1. Effect of drugs on airway immediate and late phase response to inhaled antigen

Under cover of 10 mg/kg i.p. mepyramine guinea pigs exhibited still a pronounced immediate reaction.This immediate response peaked between 5–15 min after antigen provocation. There was no significant difference in the peak immediate response between the group of animals which subsequently developed a late phase reaction, compared to those of non late phase responders, though decrease of tidal volume tended to be more pronounced in late phase responders (Fig. 2).

During a screening phase about 75% of guinea pigs demonstrated a late phase decrease of tidal volume between 4–10 h after ovalbumin (Fig. 2). 17 (25%) out of the 68 guinea pigs investigated did not have a late phase response according to the defined criteria. In addition eight sham-sensitised- and sham-challenged guinea pigs did not exhibit a late phase response. The tidal volume of animals with a late phase response was significantly lower compared to sham sensitised/-challenged animals and the group of animals without a late phase. At 24 h after antigen challenge the tidal volume of late phase responders had returned to about 75% of baseline values before challenge (Fig. 2).

Methylprednisolone administered at 40 mg/kg p.o. 1 h before challenge did not inhibit the immediate, but the late phase response. At the time points of 6, 7, 8, 9 and 10 h after antigen-challenge tidal volume was significantly increased, compared to control phase during which the same guinea pigs responded with late phase decrease of tidal volume. At 2, 4, 5 and 24 h after antigen-inhalation tidal volume was also above ovalbumin controls in methylprednisolone treated animals, but differences were not significant (Fig. 3). Theophylline at 50 mg/kg given 1 h before ovalbumin attenuated not only the peak of the immediate reaction by about 69% ($P > 0.05$, not significant, $n = 12$), but inhibited the airway late phase response significantly ($P < 0.05$, 5–10 h, $n = 12$; Fig. 4). With the exception of the time point of 4 h, theophylline increased at all remainder time points the tidal volume significantly (Fig. 4). In contrast the cysteinyl-leukotriene receptor antagonist CGP 45715A (Iralukast) at 30 mg/kg p.o. 2 h before ovalbumine neither interfered with the peak of the immediate, nor with the late phase response at any time point (Fig. 5). Similar effects were observed for all other pulmonary function parameters analysed (not shown).

3.2. Effect on inflammatory cell influx in bronchoalveolar lavage

When bronchoalveolar lavage was performed by orotracheal route 24 h after ovalbumin inhalation, total cell count (total), eosinophils, neutrophils, macrophages and lymphocytes were significantly increased in ovalbumin inhaled controls compared to sham ($\times 10^6$/lung): total 5.8 ± 0.5, eosinophils 1.2 ± 0.2, neutrophils 2.2 ± 0.3, macrophages 1.9 ± 0.1, lymphocytes 0.04 ± 0.006 in ovalbumin treated controls ($n = 15$) vs. total 1.1 ± 0.2, eosinophils 0.14 ± 0.03, neutrophils 0.007 ± 0.003, macrophages 0.8 ± 0.15, lymphocytes 0.016 ± 0.003 in sham treated animals ($n = 5$; $P < 0.05$). Methylprednisolone reduced significantly the antigen-induced increase of total cell count and eosinophil number (Fig. 6). Neither theophylline nor the cysteinyl-

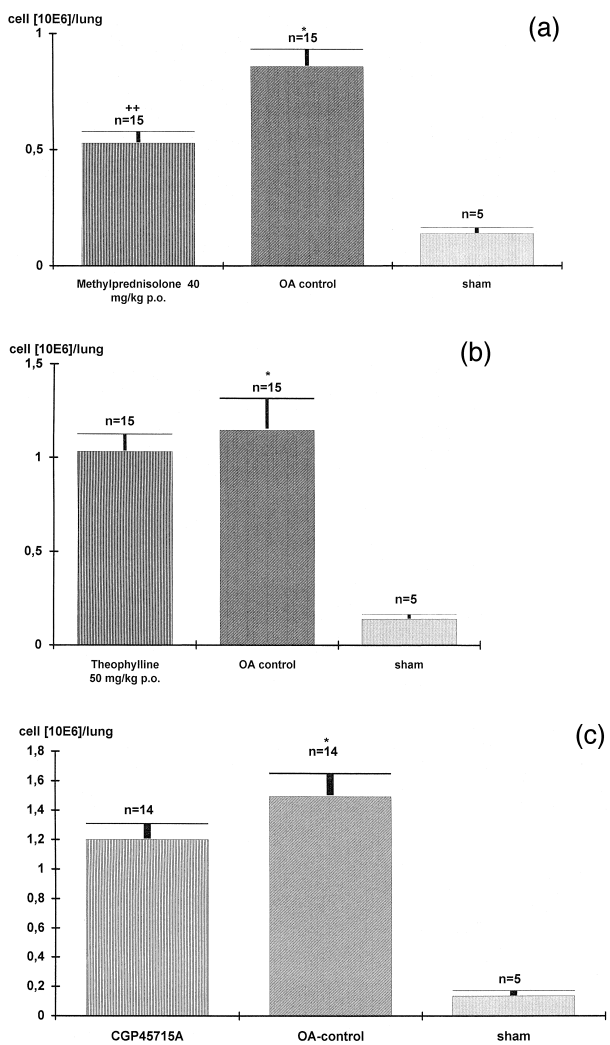

Fig. 6. Eosinophils ($\times 10^6$/lung) 24 h after ovalbumin/sham inhalation in guinea pigs. Effect of oral (a) methylprednisolone (40 mg/kg), (b) theophylline (50 mg/kg) and (c) CGP 45715A on antigen-induced eosinophil influx in cross-over study. Mean ± S.E.M. Brochoalveolar lavage was performed by 2×2 ml of saline via orotracheal route. * $P < 0.05$: significant difference of ovalbumin control to sham; ++ $P < 0.01$: significant difference between ovalbumin-control and treatment phase.

leukotriene receptor antagonist attenuated the antigen-associated total cell influx or any of the inflammatory cells analysed.

4. Discussion

The objective of the present study was to characterise a novel model for investigation of airway allergic immediate and late phase response in the conscious guinea pig by established drugs for clinical use and investigational new drugs, expected to interfere with clinical asthma. The experimental setup allowed for the first time the repeated monitoring of pulmonary function in the conscious guinea pig together with bronchoalveolar lavage for analysis of inflammatory cell influx in the same animal and to study the effect of drugs in cross-over studies.

The failure of the glucocorticoid methylprednisolone to interfere with the immediate pulmonary decrease of pulmonary function is in accordance with results in man and animals (Cockcroft and Murdock, 1987). The present study, however, did not address whether the glucocorticoid would shorten the duration of the immediate response, though the peak was not attenuated.

Inhibition of exclusively the late phase response in this novel model is in accordance with other findings in animals and man when glucocorticoids inhibited the late phase but not the immediate response (Cockcroft and Murdock, 1987).

Though incomplete, the relevant reduction of the late phase decrease of tidal volume and antigen associated eosinophil influx by the glucocorticoid suggests the inflammatory nature of the late phase response. In contrast other cell types like neutrophils were not affected by the glucocorticoid in guinea pigs under these experimental conditions. Thus, the results support some role of eosinophils in the development of antigen induced late phase response. Some correlation between the extent of the late phase response and eosinophil influx has been reported (DeMonchy et al., 1986). In other animal models (dog, Sasaki et al., 1987; rabbit, Murphy et al., 1986; monkey, Gundel et al., 1992) the extent of neutrophil accumulation correlated with the late phase response and suggested a causative involvement of neutrophils in this reaction. Although neutrophils were elevated in guinea pigs as challenged here, the lacking inhibition of its influx by methylprednisolone in contrast to inhibition of late phase response does not point to a major involvement of this cell type in the late phase reaction in this model.

The inhibition of the immediate response by theophylline is in accordance with findings in man (Pauwels et al., 1985) and in sheep (Perruchoud et al., 1984). Theophylline appears to inhibit the decreased tidal volume during the late phase reaction more pronounced than the immediate bronchoconstriction. On the other hand the incomplete inhibition of the late phase may point to additional mechanisms involved herein, resistant to inhibition by theophylline whatsoever the mechanisms of action of theophylline are.

The failure of theophylline to interfere with inflammatory cell influx in this study is in agreement with the clinical finding that theophylline does not alter the cell numbers in bronchoalveolar lavage in asthmatics as well as during the late phase response. Therefore, inhibition of cell infiltration is not a factor which would explain inhibition of the late phase reaction by theophylline. However, this does not exclude an antiinflammatory effect by theophylline during this reaction in this model, since other inflammatory reactions, like oedema formation or cell activation, have not been addressed in the present investigation.

The observation that cysteinyl-leukotrienes not only bronchoconstrict (Dahlén et al., 1980), but also increase vascular permeability (Chung and Barnes, 1992), makes them attractive mediators in the pathophysiology of asthma and therapeutic intervention thereof.

The role of cyteinyl-leukotrienes as evidenced from the effect of selective antagonists is still discussed controversial. In early clinical studies in asthmatics cysteinyl-leukotriene receptor antagonists did neither inhibit the immediate nor the late phase response (Britton et al., 1987; Bel et al., 1990) and negated a role for cysteinyl-leukotrienes in asthma. However, it remained open whether the dose which was used in these studies was adequate. In fact, recent studies with the more potent leukotriene D_4 receptor antagonist ICI 204.219 (Accolate) revealed not only inhibition of the early phase but also of the late phase in asthmatics (Taylor et al., 1991).

In contrast to clinical findings a number of animal experiments report more consistently some inhibition of immediate and late phase response (Abraham et al., 1988; Solèr et al., 1988; Sapienza et al., 1990; Foster and Chan, 1991; Nakagawa et al., 1993). Inhibition of inflammatory cell influx by cysteinyl-leukotriene receptor antagonists has been shown in some preclinical studies (Foster and Chan, 1991; Nakagawa et al., 1993), but so far not been clearly documented in clinical studies (Sapienza et al., 1990).

To investigate the role of cysteinyl-leukotrienes in this novel preclinical model of asthma, we have selected the more potent and long-acting leukotriene D_4 receptor antagonists CGP 45715A (Iralukast; Von Sprecher et al., 1991). The complete failure of adequate doses of this leukotriene D_4 receptor antagonist (Von Sprecher et al., 1991) to inhibit the peak of the immediate as well as to interfere with the late phase and the inflammatory cell influx, suggests that cysteinyl-leukotrienes such as leukotriene D_4 do not play a major role in this guinea pig model. In contrast the more or less identical pattern of immediate and late phase as well as the inflammatory cell influx between the two arms of the cross over can be taken as confirmation of the good reproducibility of the model. The

results do not appear in contradiction to the finding of inhibition of the acute response in the same species by even lower doses (Von Sprecher et al., 1991), since in the latter model guinea pigs were only sensitised once and the duration of the acute response was also considered. In contrast priming in the present model of LPR by multiple sensitisation and challenge with antigen may result in chronic inflammation and occurrence of late phase response, but also in resistance to the leukotriene D_4 receptor antagonists.

The failure of the leukotriene D_4 receptor antagonist to reduce inflammatory cell influx does not necessarily exclude an antiinflammatory effect by a leukotriene D_4 receptor antagonist, e.g. by decreasing vascular permeability and oedema formation (Chung and Barnes, 1992).

In conclusion the overall results from pharmacological intervention studies in this novel model of combined analysis of immediate, late phase response and inflammatory cell influx in bronchoalveolar lavage correlate well with clinical findings of established and investigative drugs. Therefore, this model may be useful for studying novel therapeutic principles in asthma and predict its clinical usefulness.

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