Effect of theophylline on airway responses to inhaled platelet-activating factor in man

K.F. Chung, J.W. Lammers, M. McCusker, N.M. Roberts, G.M. Nichol, P.J. Barnes


ABSTRACT: It has been suggested that theophylline may possess anti-inflammatory actions which underlie its antiasthma properties. We examined whether theophylline could inhibit the bronchoconstrictive and the bronchial hyperresponsiveness induced by inhaled platelet-activating factor (PAF) in eight nonasthmatic subjects in a double-blind, cross-over study. After oral theophylline (6 mg·kg⁻¹), plasma theophylline at 1 h was 10.4 ± 1.8 mg·ml⁻¹ (mean ± SEM) compared to 0.39 ± 0.19 mg·ml⁻¹ on the placebo day (p < 0.005). PAF, inhaled in five successive doses every 15 min, caused a 56 ± 11% fall in Vp30 (flow at 30% of vital capacity from a partial expiratory manoeuvre) after the first dose at 5 min, and diminishing responses with successive doses. Theophylline had no significant effect on PAF-induced bronchoconstriction. PAF caused a significant decrease in PC₁₅₀ (the concentration of methacholine needed to cause 40% fall in baseline Vp30) from a baseline of 12.8 mg·ml⁻¹ (geometric standard error of mean (GSEM) 1.98) to 7.9 (1.79) mg·ml⁻¹ on day 3 and 6.9 (1.74) on day 7 (p < 0.02). There was no significant difference when mean PC₁₅₀ values on corresponding days after PAF were compared between placebo and theophylline treatment periods. Our results suggest that theophylline has negligible influence on the airway effects of PAF.

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Theophylline is an effective bronchodilator, both in vivo and in vitro, but the mechanisms underlying its effect remain unclear [1]. It has been suggested that theophylline could also possess anti-inflammatory properties which may underlie some of its beneficial effects in the treatment of asthma [1]. Several recent studies would seem to support this proposal. Theophylline at therapeutic concentrations inhibited the late phase bronchoconstrictor response to allergen in allergic asthmatic subjects without affecting the acute response [2]. Another report showed similar results in workers sensitized to toluene diisocyanate subsequently exposed to the agent [3]. Because the late response is probably secondary to inflammatory events in the airways, such as airway mucosal oedema and eosinophil infiltration [4], these studies suggest that theophylline may possess anti-inflammatory properties. However, there are conflicting data on the effects of theophylline on the in vitro activation of human neutrophils which have been implicated in animal models of late-phase responses and bronchial hyperresponsiveness [5]. One study showed that theophylline inhibited the generation and release of inflammatory mediators [6], but another demonstrated a potentially effect [7].

To further study the potential effect of theophylline as an anti-inflammatory agent, we investigated whether theophylline could protect against the bronchoconstriction and bronchial hyperresponsiveness induced by platelet-activating factor (PAF) in man. PAF is a potent inflammatory mediator which can mimic several features of the asthmatic airway such as bronchoconstriction, bronchial hyperresponsiveness, airway microvascular leakage and eosinophil chemotaxis and activation [8]. Since the increase in bronchial responsiveness after PAF inhalation is likely to be linked to inflammatory events induced by this mediator [8], any potential inhibitory effect of theophylline on this response could be the result of an anti-inflammatory mechanism. In addition, part of the acute bronchoconstrictor effect of PAF could be secondary to the development of airway oedema, resulting from an increase in airway microvascular leakage [9]. In a preliminary report, theophylline has been shown to inhibit the increased microvascular permeability induced by PAF administered intratracheally in the guinea-pig [10].

Methods

Subjects

Eight normal, healthy non-asthmatic adults (7 males) aged 22–34 yrs (mean 24.8 yrs) agreed to participate in...
the study, which was approved by the Ethics Committee of the National Heart and Chest Hospitals. All subjects gave informed consent. One subject was a smoker and two were atop with positive skin prick tests to several common allergens and seasonal symptoms of hay fever. At the start of each study period, all subjects had been free of upper respiratory tract infections for at least 4 weeks. Baseline forced expiratory volume in one second (FEV₁) for each subject was within 95% of the value predicted for age and height. None of the subjects were taking any medication. Caffeine-containing beverages were withheld for 2 h prior to challenge on each day.

Study design

A double-blind, randomized, cross-over study was designed to compare the effects of plain theophylline against matched placebo (Riker Laboratories, Loughborough, UK). Each subject was studied during two periods separated by at least four weeks. During each period, the subject attended the laboratory on five occasions over an eight to nine day period. Airway responsiveness to methacholine was first measured and one to two days later the subject inhaled PAF. Responsiveness to methacholine was measured at the same time of day one, three and seven days later. This protocol was chosen on the basis of a previous study, which demonstrated that the maximum increase in responsiveness occurred at three days after PAF inhalation and, in some subjects, persisted for up to 4 weeks [11].

The oral dose of theophylline administered was approximately 6 mg·kg⁻¹ body weight. Each subject had a trial run on that dose when serum theophylline level was assayed at 1 h after ingestion. The dose of theophylline was adjusted accordingly for the study period. Theophylline was administered one hour prior to PAF challenge for each study period. Venous blood samples were obtained one hour after the tablets were taken and stored for determination of theophylline levels by fluorimnunoassay at a later date. Thus, theophylline blood levels were within therapeutic levels only at the time of PAF challenges and not during any of the methacholine challenges.

PAF inhalation challenge

PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine: Novabiochem AG, Switzerland) was stored in 100% ethanol at -20°C at a concentration of 10 mg·ml⁻¹. On the study day, a 1.5 mg·ml⁻¹ solution was prepared in 0.9% saline containing a final concentration of 0.03% heat-treated human serum albumin. The diluent had no effect on baseline airway function. PAF aerosol was delivered from a nebulizer attached to a dosimeter (MEFAR, Brescia, Italy) driven by compressed air at a pressure of 22 psi. On each nebulization, which lasted for 10 s, the subject inhaled for 3 s from functional residual capacity to total capacity and held his breath for 7 s before breathing out normally. The output of the nebulizer was 6 µl per breath. Because of the development of rapid tachyphylaxis of the bronchoconstrictor response to PAF, we administered equal doses of PAF (18 µg) every 15 min on five occasions (total dose=90 µg).

The response to PAF was measured from volume-standardized partial expiratory flow-volume curves [12]. Partial curves were used as a more sensitive measure of bronchoconstriction, thus reducing the necessity for administering large doses of PAF or methacholine. Flow-volume curves were measured using a rolling spirometer (Vitalograph, UK) and were analysed using a Hewlett-Packard microcomputer (Collingwood Measurements, Leicester, UK). The flow-volume curves were stored and could be displayed after each manoeuvre. Subjects initially performed a full vital capacity, and the measurement of flow was then made at 30% of vital capacity, measured from total lung capacity (Vₚ₃₀). Flow volume manoeuvres were performed by expiration from just above tidal inspiration to residual volume, followed by inhalation to total lung capacity before breathing out normally. All subjects were fully trained in these breathing manoeuvres before entering the study. Measurements of Vₚ₃₀ were made at 1, 3, 5, 10 and 15 min after each inhalation of PAF. The responses of Vₚ₃₀ were expressed as the percentage fall from the baseline Vₚ₃₀ measurement.

Methacholine challenge

Methacholine chloride solutions (Sigma, UK) were prepared in 0.9% sodium chloride (NaCl) and stored at 4°C. Prior to use, they were allowed to warm to room temperature. Increasing doubling concentrations, ranging from 0.125–64 mg·ml⁻¹, were used. After three baseline values of Vₚ₃₀ had been obtained, the subject inhaled five breaths of 0.9% NaCl from the dosimeter. Vₚ₃₀ was measured at 90 and 150 s and the mean Vₚ₃₀ obtained. Starting at a concentration of methacholine determined on the basis of prior assessment of airway responsiveness, these steps were repeated and successive doubling concentrations (five breaths) administered every three min until approximately 50% reduction of Vₚ₃₀ had occurred. The provocative concentration of methacholine needed to cause a 40% fall in Vₚ₃₀ (PC₁₄₀) was then computed.

Data analysis

PC₁₄₀ values were log-transformed before analysis, and geometric mean and geometric standard errors of mean (Gstem) calculated. All other data have been reported as the mean and standard error. In order to determine the effect of PAF on airways responsiveness, a two-factor analysis of variance and the Newman-Keuls multiple range test were performed [13]. To determine whether theophylline had an effect on PAF responses, a paired t-test was used to compare the values obtained on the theophylline and placebo days.
THEOPHYLLINE AND AIRWAY RESPONSE TO PAF

Fig. 1. - Effect of placebo (○) and of theophylline (●) on the bronchoconstrictor responses to inhaled PAF. Airway calibre was measured as \( V_{p_{30}} \) (expiratory flow rate at 30% of the vital capacity measured from a partial expiratory manoeuvre). Similar doses of PAF (18 \( \mu \text{mol} \)) were inhaled every 15 min. Theophylline had no significant effect on PAF-induced bronchoconstriction. Data are shown as mean±SEM.

**Results**

On the theophylline day, mean serum theophylline level 1 h after ingestion of theophylline tablets was 10.4±1.8 mg·ml\(^{-1}\) compared to 0.39±0.19 mg·ml\(^{-1}\) on the placebo day (\( p<0.005 \)). PAF inhalation induced a transient facial flushing in all subjects within 3–5 min, associated with chest tightness and coughing; this effect was not seen with successive inhalations. Theophylline had no effect on these symptoms. Mean baseline \( V_{p_{30}} \) on placebo days was 149±16 l·min\(^{-1}\), compared to 146±12 l·min\(^{-1}\) on the theophylline day. The first inhalation of PAF induced a maximum fall of 56±11% in \( V_{p_{30}} \) at 5 min (\( p<0.01 \)), and there was rapid tachyphylaxis with the subsequent doses (fig. 1). On the theophylline day, there was a tendency for the mean fall in \( V_{p_{30}} \) to be less when compared to the responses on the placebo day, but this was not statistically significant at any time-point (fig. 1). PAF still caused a significant fall of 45±9% in \( V_{p_{30}} \) at 5 min after the first inhalation (\( p<0.01 \)).

After placebo, PAF caused a significant increase in bronchial responsiveness on subsequent days. Thus, mean baseline \( P_{C_{40}} \) to methacholine for the eight subjects was 12.8 mg·ml\(^{-1}\) (GSEM 1.98); this fell to 7.9 (GSEM 1.79) on day 3 and 6.9 (GSEM 1.74) on day 7 (\( F \) value=5.30; \( p<0.02 \)). Mean \( P_{C_{40}} \) after placebo was not significantly different from mean \( P_{C_{40}} \) after theophylline on the corresponding days following PAF exposure. Two subjects showed no change in bronchial responsiveness after PAF (fig. 2) and, even when the results for the six other subjects were examined, there was no significant difference in the increase in bronchial responsiveness observed after PAF during the placebo and theophylline days (fig. 3). Thus, for these subjects after placebo, mean \( P_{C_{40}} \) decreased from 15.0 mg·ml\(^{-1}\) (2.05) to 6.9 mg·ml\(^{-1}\) (1.85) on day 3 (\( F \) value=14.1; \( p<0.001 \)); after theophylline, mean \( P_{C_{40}} \) decreased from 13.6 mg·ml\(^{-1}\) (1.82) to 5.2 mg·ml\(^{-1}\) (2.06) on day 3 (\( F \) value=4.2; \( p<0.05 \)).

Fig. 2. - Individual changes in \( P_{C_{40}} \) (concentration of methacholine needed to cause a 40% fall in baseline \( V_{p_{30}} \)) for eight normal subjects at three days after PAF following pretreatment with placebo (○) or theophylline (●). The two subjects who showed no changes in \( P_{C_{40}} \) values during placebo and theophylline periods were the same. Data are shown as mean±SEM.
In the present study, we found that theophylline administered at a dose that produces therapeutic blood levels during PAF challenge did not have any significant effect on the bronchoconstriction induced by inhaled PAF. In addition, in the six subjects in whom bronchial responsiveness to methacholine increased, there was no effect of theophylline on PAF-induced airway hyperresponsiveness. One of the advantages of using normal subjects is that there is no question of prior anti-asthma therapy such as beta-adrenergic agonists which may influence the responses we are studying. In addition, asthmatic subjects do not show an increase in bronchial responsiveness after PAF exposure [14].

The mechanisms by which PAF induces an increase in bronchial responsiveness are unclear but, because PAF is rapidly metabolized within the airways [15], it is likely that PAF causes a train of events within the airways leading to hyperresponsiveness [8]. PAF is one of the most potent inducers of human eosinophil chemotaxis in vitro [16] and has been shown to cause recruitment of eosinophils within the airways of guinea-pigs and baboons [17, 18]. Eosinophils, which are prominent within the airways of asthmatic subjects, have been implicated in the pathogenesis of the bronchial hyperresponsiveness of asthma [19]. Therefore, any beneficial effect of theophylline could have been a reflection of an inhibitory action on the activation of eosinophils. In a recent study from our laboratory, we showed that within the range of therapeutic concentrations, theophylline (10⁻⁴–10⁻³ M) had a small enhancing effect on the release of superoxide anions from guinea-pig and human eosinophils stimulated by zymosan in vitro [20]. This observation is consistent with that of Schumacher and Thomas [7] who found an enhancement of neutrophil aggregation, lysosomal enzyme release and superoxide anion formation by theophylline. Thus, if bronchial hyperresponsiveness is a reflection of eosinophil or neutrophil recruitment and activation by PAF, theophylline would not be expected to inhibit PAF-induced hyperresponsiveness, as we have observed in the present study. Similarly, DuToit et al. [21] found that long-term theophylline administration had no significant effect on the chronic established bronchial hyperresponsiveness of asthmatic subjects.

Our results are also in agreement with those of Map et al. [3] who showed that theophylline had no effect on bronchial hyperresponsiveness induced by toluene diisocyanate in sensitized workers. However, the late response was inhibited [3], in agreement with the report of Paulwells et al. [2] who studied the late response to common allergens. Although the late-phase response after allergen challenge has been temporally associated with the presence of eosinophils in bronchoalveolar lavage fluid [22], it is unlikely that the beneficial effect of theophylline in these studies is explained on the basis of an inhibitory effect on eosinophil activation in view of the in vitro potentiating effects of theophylline as discussed above [8].

It is also possible that the airways obstruction observed during the late response is secondary to airway macosal oedema resulting from increased vascular permeability. In the guinea-pig, theophylline has been shown to be effective in inhibiting both the increased airway microvascular leakage induced by immunoglobulin E (IgE) mediated mechanisms and by PAF [10, 23]. However, another study from our own laboratory showed no significant inhibitory effect of theophylline against PAF-induced airway microvascular leakage in the same species [24]. It is possible that the fall in Vp₃₀ after PAF inhalation may be due to airway narrowing due to airway oedema. Firstly, there is little correlation between the airway responsiveness to PAF and that to methacholine, which acts mainly by causing airway smooth muscle contraction [12]. Secondly, asthmatic subjects who are hyperresponsive to methacholine respond to PAF to a similar extent as do normal subjects [14]. Thirdly, salbutamol, a β₂-adrenergic agonist, only partially inhibits PAF-induced bronchoconstriction [9]. In this respect some inhibitory effect of theophylline on the PAF-induced falls in Vp₃₀ might be expected. However, the small reduction in the falls in Vp₃₀ due to PAF after theophylline

**Fig 3.** Mean PC₃⁰ for 6 of the 8 subjects (excluding the two who did not respond to PAF) 0-7 days after PAF inhalation following placebo (○) and theophylline (●). There were significant increases in bronchial responsiveness during both study periods; for placebo, p<0.001; for theophylline, p<0.05. There was no significant difference between mean PC₃⁰ on the corresponding days after PAF for the two study periods.
treatment was not statistically significant. Because theophylline is a functional antagonist of several constrictor agents [1], our negative results again reinforce the fact that PAF may not have any direct airway smooth muscle constrictor effects.

Although theophylline does not appear to influence the effects of PAF on the airways in normal subjects, it does not exclude the possibility that it may inhibit the effects of other putative mediators involved in the pathogenesis of asthma. Thus, it is possible that the inhibitory effect of theophylline against the late-responses observed after allergen or toluene disocyanate [2, 3] may be specific for inflammatory mediator(s) involved in this particular inflammatory process.

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References


RÉSUMÉ: L'on a suggéré que la théophylline pourrait posséder une activité anti-inflammatoire, qui serait à la base de ses propriétés anti-asthmatisantes. Nous avons examiné si la théophylline pouvait inhiber la broncho-constriction et l’hyperréactivité bronchique induites par le facteur activateur des plaquettes (PAF) en inhalation, chez huit sujets non-asthmatiques, au cours d’une étude en double aveugle avec permutation croisée. Après administration de théophylline par voie orale (6 mg·kg⁻¹), le taux de théophylline plasmatique après 1 heure était de 10.4±1.8 mg·ml⁻¹ (moyenne±SEM), par comparaison à 0.39±0.19 mg·ml⁻¹ le jour placebo (p<0.05). Le PAF inhalé à 5 doses successives pendant 15 minutes a provoqué une chute de 56±11% du Vₐₐₐₐ (début à 30% de la capacité vitale au cours d’une manœuvre expiratoire partielle) après la première dose à 5 minutes, avec des réponses diminuant au cours des doses successives. La théophylline n’a pas eu d’effet significatif sur la bronchoconstriction induite par le PAF. Le PAF a causé une diminution significative de PC₁₅₀ (la concentration de méthacholine nécessaire pour provoquer une chute de 40% du Vₐₐₐₐ de base). Cette diminution de PC₁₅₀ passe de 12.8 mg·ml⁻¹ (GSEM 1.98 à l’état basale) à 7.9 (1.79 mg·ml⁻¹) le jour 3, et 6.9 (1.74) le jour 7 (p<0.02). Il n’y avait pas de différence significative entre les périodes de traitement par placebo et par théophylline en ce qui concerne les valeurs moyennes de PC₁₅₀ aux jours correspondants après PAF. La théophylline n’a pas eu d’effet significatif sur l’hyperréactivité bronchique induite par le PAF.
lorsque l'analyse a été réalisée chez 6 des 8 sujets qui montraient individuellement des diminutions du $P_{C_{02}}$ après PAF. Nos résultats suggèrent que la théophylline n'a que des effets négligeables contre l'action du PAF sur les voies aériennes, action que pourrait être médiane par le recrutement des cellules inflammatoires et par l'augmentation de la perméabilité microvasculaire des voies aériennes.

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