

ORIGINAL PAPER

Targeting adenosine receptors in the treatment of allergic rhinitis: a randomized, double-blind, placebo-controlled study

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Clinical and Experimental Allergy

Summary

Background There is evidence that adenosine plays a role in the pathogenesis of asthma and rhinitis; however, it is currently unclear whether adenosine receptors are useful therapeutic targets in the treatment of allergic airway diseases.

Objective The study evaluated the efficacy of intranasal treatment with an adenosine A_{2A} receptor agonist/adenosine A_3 receptor antagonist (50 µg), administered twice daily for 7 days, to reduce nasal symptoms and release of inflammatory mediators following intranasal allergen challenge in patients with allergic rhinitis (AR). The compound was compared with twice-daily treatment with intranasal fluticasone propionate nasal spray (FPANS) for 7 days.

Methods A randomized, double-blind, double-dummy, placebo-controlled, three-way balanced, incomplete block, crossover study was conducted on 48 males with verified AR.

Following intranasal challenge with either an extract from the house dust mite (HDM), *Dermatophagoides pteronyssinus*, rye grass or cat dander, nasal responses and the concentrations of albumin, tryptase, myeloperoxidase, eosinophilic cationic protein, epithelial neutrophil-activating protein-78 (ENA-78), IL-5 and IL-8 in nasal secretions were measured and treatment groups were compared.

Results Drug improved nasal blockage but had no significant effect on rhinorrhoea, number of sneezes or peak nasal inspiratory flow measurements when compared with placebo. Drug reduced tryptase release after EAR but did not significantly reduce the levels of other mediators.

Conclusion A novel agonist/antagonist of adenosine A_{2A} and A_3 receptors appears to have limited clinical benefit in both the early-phase and the late-phase response to intranasal allergen challenge. However, reduction of some pro-inflammatory mediators suggests that comparable, more selective compounds may have additional benefits meriting further investigation.

Keywords A_{2A} agonist, A_3 antagonist, adenosine receptor, allergic rhinitis, clinical trial, inflammatory mediators

Submitted 1 July 2005; **revised** 15 May 2006; **accepted**: 5 May 2006

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Introduction

Adenosine is a naturally occurring purine nucleoside that is generated following the cleavage of intracellular adenosine monophosphate (AMP) as it encounters the cell membrane-associated 5'-nucleotidase. Adenosine levels are increased during inflammatory conditions in the airways. The exact cellular sources of adenosine are currently unclear; however, mast cells, neutrophils and platelets have been suggested as putative sources [1].

There is accumulating evidence that adenosine plays a role in the pathogenesis of asthma and allergic rhinitis (AR). For example, elevated adenosine levels have been detected in bronchoalveolar lavage fluid obtained from asthmatic subjects [2], and inhaled adenosine has been shown to cause bronchoconstriction in both asthmatics and AR, but not in healthy individuals [3, 4]. Furthermore, intranasal challenge with the related nucleotide, 5'-AMP, resulted in more severe nasal symptoms in subjects with AR than in healthy subjects. Finally, significantly higher

levels of histamine have been measured in nasal lavage fluids from allergic subjects but not from healthy controls following intranasal AMP provocation [5]. Taken together, these findings suggest that modulating adenosine in the airways may be of therapeutic benefit in subjects with asthma and AR.

The actions of adenosine are mediated by interactions with specific cell surface receptors. To date, four adenosine receptor subtypes have been identified, designated A₁, A_{2A}, A_{2B} and A₃. These receptors are all expressed in human airway tissue [6]. Adenosine A_{2A} receptors are also expressed on mast cells, eosinophils, neutrophils, monocytes, macrophages and lymphocytes. In general, activation of A_{2A} receptors on inflammatory cells results in anti-inflammatory effects including suppressing the adherence of neutrophils to the endothelium [7], down-regulating integrin expression [8] and inhibiting neutrophil degranulation [9]. A_{2A} receptor activation can also result in a decrease in histamine and tryptase release from mast cells [10]. The A₃ adenosine receptor subtype appears to have conflicting roles in airway inflammatory disease, with stimulation of receptors on mast cells inducing histamine release, but activation of receptors on eosinophils leading to the suppression of chemotaxis [10], degranulation and superoxide anion release [11]. The cellular localization of adenosine A₃ receptors is less well defined than the A_{2A} subtype but they have been shown to be present on human eosinophils [12]. These findings suggest that both A_{2A} agonists and A₃ antagonists may have activities appropriate for the treatment of airway inflammatory diseases.

(2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-{[(1*S*)-2-hydroxy-1-(phenylmethyl)ethyl]amino}-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol (2*Z*)-2-butenedioate (salt) is a novel, potent and selective agonist of the human A_{2A} receptor and antagonist of the human A₃ receptor (Butchers *et al.*, submitted for publication). Preliminary data from animal models demonstrated that this compound potently inhibits the infiltration of eosinophils and neutrophils into the lung following challenge with antigen or lipopolysaccharide (GSK data on file). It was therefore proposed that this compound might act as a non-steroidal anti-inflammatory compound that could be used for the treatment of asthma, AR and chronic obstructive pulmonary disorder. In this study, the effects of this compound on nasal symptoms, nasal patency and mediator release following nasal allergen challenge in individuals with AR were assessed.

Materials and methods

Study design

This randomized, double-blind, double-dummy, placebo-controlled, three-way balanced incomplete block, cross-

over study was conducted at four sites in Australia and New Zealand out of the pollen season between June and September 2002. Forty-eight out of 68 male subjects ranging in age from 18 to 55 years with persistent AR were found to meet the study eligibility criteria. Before the study commencing, approval was obtained from the relevant Human Research Ethics Committees in Sydney, Melbourne and Christchurch, and written, informed consent was obtained from each of the subjects.

The study consisted of a screening visit within 3 weeks of the first dose, three treatment periods of 7 days each, separated by washout periods of at least 2 weeks, and a follow-up visit, 7–14 days after the last dose. Subjects were randomized in accordance with the balanced, incomplete block randomization schedule to receive three of the four intranasal medications: drug (50 µg) or its matched unscented placebo, fluticasone propionate nasal spray (FPANS) (200 µg) or its matched scented placebo. The researchers were blinded to the randomization process and the drug administration. Thirty-six out of 37 volunteers completed drug, 36/36 FPANS and 36/37 and 35/36 the respective placebos. The three withdrawals were for reasons other than side effects or lack of efficacy.

Patient selection

All subjects had a clinical history of AR and at least one positive skin prick test (SPT) response (≥ 4 mm weal) to house dust mite (HDM), rye grass or cat dander. In addition, each subject exhibited a positive clinical nasal response to one of these allergens following nasal challenge, as judged by a minimum of five sneezes within 10 min of allergen challenge or ≥ 5 point increase on the Lebel Symptom scoring system [13]. Patients were excluded from the study if they had a history of upper respiratory infection within 4 weeks of the study; had current signs or symptoms of rhinitis; had an anatomical obstruction; had a history of smoking in the previous 6 months; had received immunotherapy in the past 3 years; or had used any of the following drugs within the specified time period: systemic corticosteroids within the last two months, intranasal steroids in the last four weeks, inhaled or intranasal sodium cromoglycate or nedocromil within the last 3 weeks; and short- or long-acting anti-histamines within 5 days of allergen challenge. Patients were allowed to continue with previously prescribed fluticasone dipropionate (≤ 250 mg) or beclomethasone dipropionate (≤ 500 mg); they were on no other medications.

Nasal allergen challenge and sample collection

Following administration of the intranasal medication twice-daily for 6 days, baseline data were collected. One hour after the morning dose of medication on the seventh

day, intranasal allergen challenge (2000 allergen units (AU) per nostril; Hollister-Stier Laboratories, Spokane, WA, USA) was performed using the allergen that had generated the largest weal size during the SPT conducted at the screening visit. Nasal symptoms and peak nasal inspiratory flow (PNIF) were measured 10, 30 min, 1, 1.5, 2, 3, 3.5 h and then every hour until 10.5 h after allergen challenge. Nasal lavage was performed 1, 4, 9 and 24 h post-allergen challenge. Sampling of nasal secretions

using a nasal filter paper was performed 1, 3, 8 and 24 h after allergen challenge.

Outcome measurements

The primary outcome measure was the mean total nasal symptom score over 10 min to 10.5 h post-challenge. This was evaluated with a categorical scoring system recording the prevalence and severity of nasal blockage, sneezing

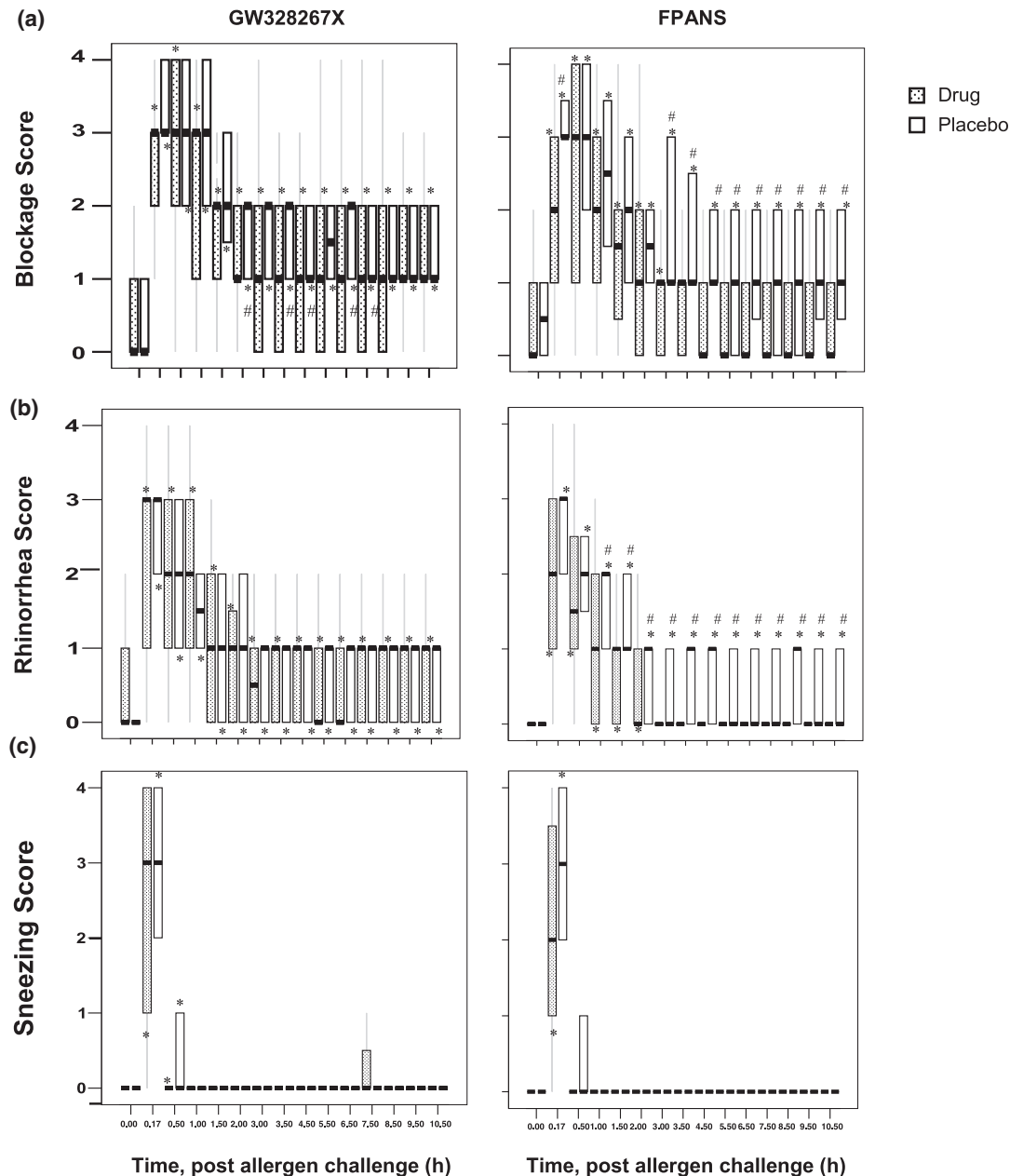


Fig. 1. Effects of drug and fluticasone propionate nasal spray (FPANS) on clinical symptoms and peak nasal inspiratory flow rates following nasal allergen challenge. Subjects were required to rate the severity of their nasal symptoms including blockage, sneezing and rhinorrhoea both before and after treatment with drug, unscented placebo, FPANS or scented placebo using a categorical scoring system. Data are expressed as median $\pm$ interquartile range ($n = 36$). * $P < 0.05$ compared with baseline; # $P < 0.05$ compared with treatment with placebo.

and rhinorrhoea. Subjects rated their symptoms on a five-point scale where 0 represented none; 1, mild; 2, moderate; 3, severe; and 4, very severe.

Secondary outcome measures were the mean PNIF score from 10 min to 10.5 h, mediator and cytokine levels in nasal secretions, cell counts in nasal brush samples, safety and general, nasal tolerability of the drug.

PNIF was measured using an In-Check portable nasal inspiratory flow meter (Clement Clarke International, Harlow, Essex, UK) with the best of three efforts being used.

Nasal lavage was performed using a nasal pool device [14]. Sampling of the nasal fluid using filter paper was performed according to the method described by Linden et al. [15]. Epithelial neutrophil-activating protein-78 (ENA-78) in nasal lavage fluid samples and IL-5 and IL-8 in nasal filter paper eluate samples were measured using commercially available, specific enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA). Myeloperoxidase (MPO) levels in nasal lavage fluid were measured at Simbec Research Limited (Merthyr Tydfil, Wales, UK) using a commercially available enzyme immunoassay (EIA) kit (Immunodiagnostik AG, Bensheim, Germany). Mast cell tryptase and eosinophil cationic protein (ECP) were measured in nasal lavage fluid using a UniCAP[®] system fluoroenzyme immunoassay (FEIA) (Pharmacia Diagnostics; Uppsala, Sweden), according to the manufacturer's instructions. Levels of albumin in nasal lavage fluid were determined by nephelometry using the Beckman IMMAGE ALB test (Beckman Coulter Inc.; Fullerton, CA, USA). Nasal brush samples were examined for eosinophil numbers but yielded erratic results and were not included in the study results. Safety and tolerability were recorded in a daily symptom diary and reviewed at each visit.

Statistical analysis

No completed subject data were excluded from analysis. At the design of the trial, a sample size of 48 evaluable

subjects was calculated to provide at least 90% power to detect a 20% change in mean categorical total nasal symptom score between 10 min and 10.5 h post-allergen challenge at the 5% two-sided significance level. Calculations were performed using Microsoft Excel and Analyse-It software (Analyse-It Limited; Leeds, UK). To assess differences in symptom scores and PNIF measurements between the four treatment groups, the area under the curve (AUC) was calculated and compared using the Wilcoxon signed-rank test. Mediator release was reported as median $\pm$ interquartile range. The Wilcoxon signed-rank test was also used to compare the effects of drug and FPANS on mediator release at each time-point. *P* values < 0.05 were considered to be statistically significant.

Results

All subjects enrolled (48) completed the study. The mean age was 31.3 ± 9.3 years, and the age range was 18–50 years. HDM challenge was performed in 28 subjects, rye grass challenge in 18 and challenge with cat dander in two persons.

Measurements of symptoms, PNIF and nasal mediators on placebo days matched with drug and with FPANS were virtually identical (Figs 1–3). In each of the treatment groups, a statistically significant increase in the severity of nasal blockage, rhinorrhoea and sneezing occurred within 10 min of intranasal allergen challenge (Figs 1a–c). The degree of nasal obstruction, determined by PNIF, also increased (Fig. 2). Drug slightly improved nasal blockage but had no significant effect on PNIF measurements, rhinorrhoea or the number of sneezes when compared with the matched placebo (Fig. 1). FPANS treatment, however, significantly improved nasal blockage scores, PNIF measurements and rhinorrhoea. As with drug, FPANS had no effect on the number of sneezes.

When the effects of drug and FPANS were expressed as AUC, a clear, significant difference in AUC for blockage was found between FPANS and placebo. Only a marginal

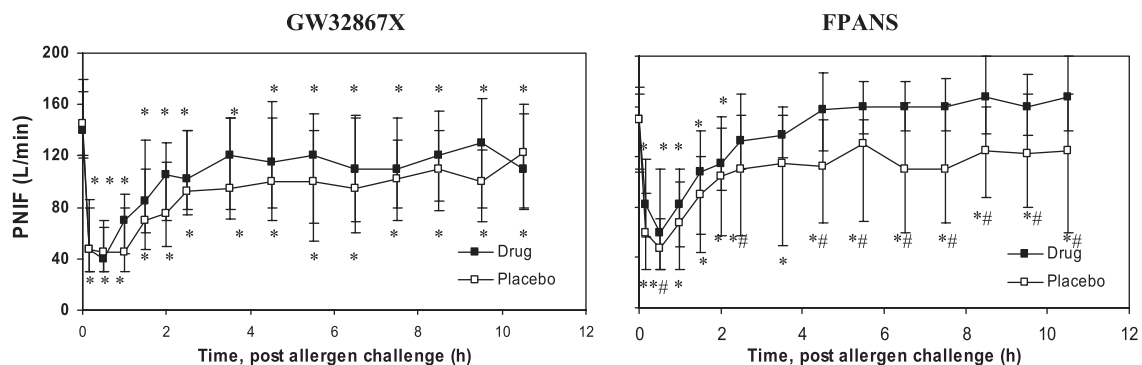


Fig. 2. Effects of drug and fluticasone propionate nasal spray (FPANS) on peak nasal inspiratory flow rates following nasal allergen challenge. Peak nasal inspiratory flow (PNIF) rates were measured both before and after treatment with drug, unscented placebo, FPANS or scented placebo. Data are presented as median $\pm$ IQ range ($n = 36$). **P* < 0.05 compared with baseline; #*P* < 0.05 compared with treatment with placebo.

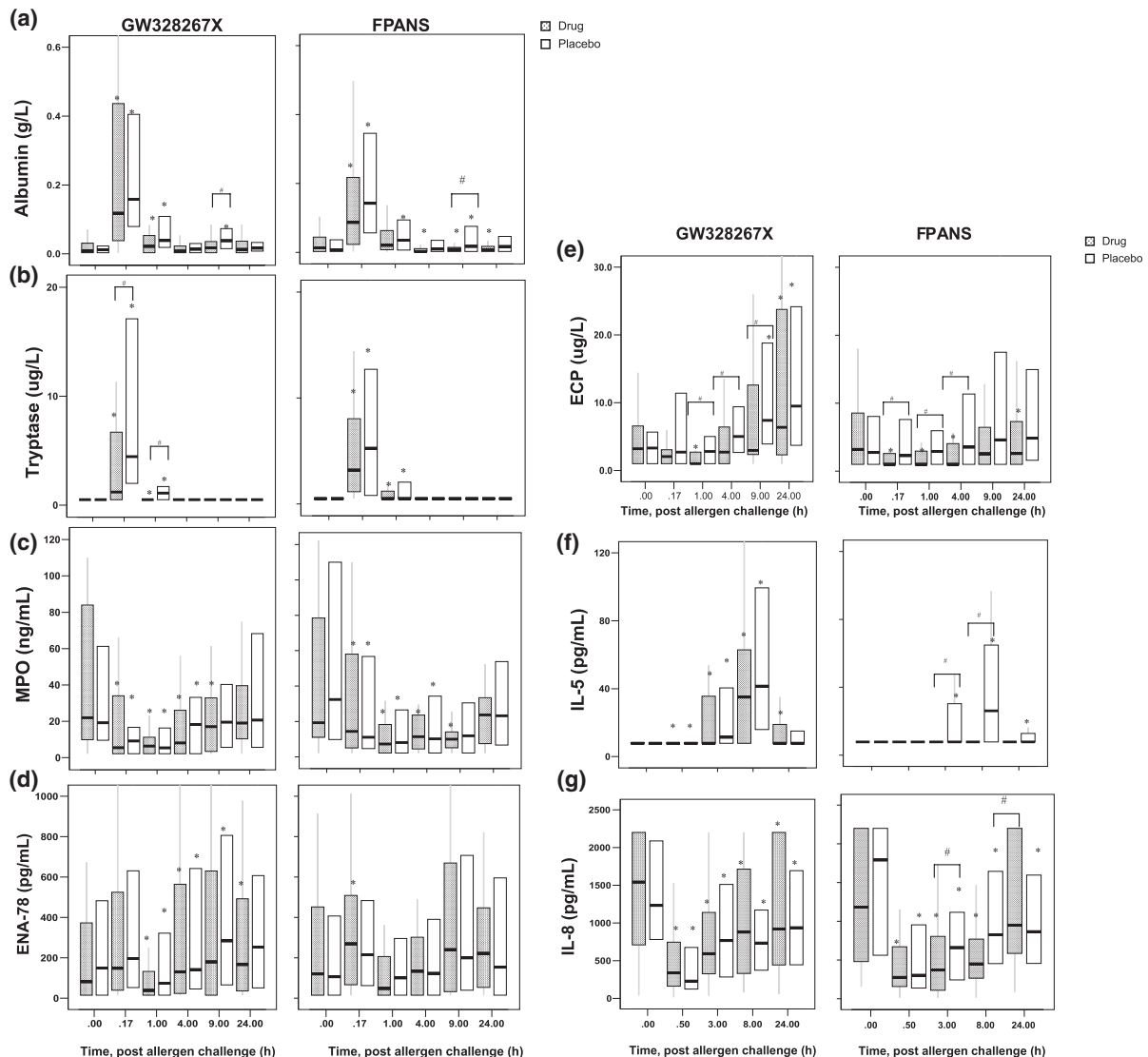


Fig. 3. Effects of drug and FPANS on mediator release following nasal allergen challenge. Albumin (a), tryptase (b), MPO (c), ECP (d) and IL-5 (e) concentrations in nasal lavage fluid were measured 10 min, 1, 4, 9 and 24 h after allergen challenge. IL-5 (f) and IL-8 (g) levels in nasal filter paper eluate were determined at 0.5, 1, 3, 8 and 24 h post-allergen challenge. The results are presented as median $\pm$ interquartile range ($n = 36$). * $P < 0.05$ compared with baseline; # $P < 0.05$ compared with treatment with placebo.

difference was noted between drug and placebo (Table 1). There was no significant difference in the AUC for rhinorrhoea, sneezing or PNIF measurements following drug treatment compared with placebo or in the AUC for sneezing following FPANS treatment. There were, however, significant differences in AUC for rhinorrhoea and PNIF measurements following FPANS treatment compared with placebo.

Neither drug nor FPANS treatment had any significant effect on the levels of albumin, MPO or IL-5 (Figs 3a, c and d). However, drug reduced tryptase early at 10 and 60 min after allergen challenge (Fig. 3b). FPANS treatment resulted in a significant decrease in ECP release at 10 min, 1 and 4 h after allergen challenge, while drug reduced ECP 1, 4 and 9 h after allergen (Fig. 3e). These changes were

not matched by changes in eosinophil numbers (data not shown). FPANS also reduced IL-5 and IL-8 production 3 and 8 h after allergen challenge. In contrast, drug did not influence IL-5 or IL-8 release (Figs 3f and g).

Discussion

Intranasal administration of a selective adenosine A_{2A} receptor agonist A_3 receptor antagonist produced limited benefits on the classic clinical symptoms of AR, nasal airflow measurements or mediator release following nasal allergen challenge. The results from this study using a compound with agonist/antagonist activities suggest that the adenosine A_{2A} and A_3 receptors may play only a limited role in nasal responses following intranasal

Table 1. Area-under-the curve (AUC) following nasal allergen challenge

AUC*	Drug	Unscented placebo	P-value	FPANS	Scented placebo	P-value
Blockage	13.9 ± 2.8	18.3 ± 2.8	0.0346	8.9 ± 2.3	16.4 ± 3.5	< 0.0001
Rhinorrhea	8.9 ± 2.3	9.6 ± 7.9	0.6945	3.5 ± 1.1	9.3 ± 2.5	0.0006
Sneezing	3.0 ± 1.6	2.5 ± 0.9	0.6464	1.0 ± 0.5	2.1 ± 1.1	0.1460
PNIF	1164 ± 159	1042 ± 162	0.1717	1474 ± 123	1136 ± 164	0.0033

*AUC was calculated for blockage, rhinorrhoea, sneezing and PNIF from baseline up to 10.5 h following intranasal allergen challenge.

allergen challenge and hence in the genesis of allergen-associated nasal inflammation. However, these conclusions must be guarded because an approach using pharmacological ligands has a number of shortcomings.

AR is a chronic inflammatory condition of the nose that affects 5–50% of the population worldwide [16]. The nasal allergen challenge model is an established technique that is widely used to study disease mechanisms and to assess new therapeutic agents. This model differs from natural exposure models (seasonal studies and exposure chamber studies) particularly in the amount of allergen exposure; however, its main advantage is its reliability and ease of administration. Our study was conducted in patients with AR whose diagnosis was based on the characteristic clinical history and the development of nasal symptoms and signs of significant nasal obstruction as determined by PNIF following allergen provocation [17]. The changes reflected a nasal response to allergen of sufficient severity to detect modulation by a therapeutic agent – for example PNIF was reduced after allergen challenge by a mean of approximately 50%. The reaction to FPANS, an intranasal topical corticosteroid, was used as a positive control and verified that the clinical and biochemical consequences of allergen challenge could be attenuated. Reductions in FPANS responses were significant and comparable in character and extent to those reported for budesonide [15]. In contrast, a novel adenosine A_{2A} receptor agonist and adenosine A₃ receptor antagonist partially decreased nasal blockage, as determined by occlusion scores, but had no significant effect on sneezing, rhinorrhoea, PNIF. The compound inhibited tryptase release during the EAR compared with placebo and ECP release 1–9 h post-challenge. FPANS was clearly more effective than the drug and significantly reduced most of the clinical manifestations of AR for approximately 10 h in this model. Intranasal corticosteroids are currently considered to be the most effective treatment for nasal allergic disease [18] and their superior activity suggests that the strategies used in the current study to target adenosine receptors will not be successful in the current format.

The pathogenesis of AR is not fully understood but it is believed that products of mast cell degranulation such as tryptase and histamine and release of chemokines including ENA-78 and IL-8, cytokines such as IL-5 and products of neutrophils (MPO) and eosinophils (ECP) all contribute to mucosal inflammation [19–21]. We measured a number

of these factors to gauge the effect of treatment on inflammatory mediators, and because a selective adenosine A_{2A} receptor agonist, CGS-21680, has previously been shown to dose-dependently reduce eosinophil, neutrophil and lymphocyte numbers in BAL fluid and decrease MPO and eosinophil peroxidase activity in ovalbumin-sensitized Brown Norway rats [22]. Release of one mediator commonly associated with the early-phase response (EPR), tryptase, was modulated by drug but not FPANS. This suggests that adenosine receptors may be implicated in early responses to allergen through actions on mast cells. Although drug was able to reduce nasal tryptase secretion that is consistent with the known effects of mast cell A_{2A} activation (suppression of IgE-mediated degranulation), this did not translate into clinical efficacy [10]. The reason for this is not clear but the pharmacologic effects of adenosine are very complex, mediated via receptor subtypes A₁, A_{2A}, A_{2B} and A₃, which are present on many cell types; human mast cell lines have been shown to co-express A_{2A} and A_{2B} receptor subtypes and the latter may be more important in allergic inflammation [1]. Significant reductions in ECP were noted following both drug and FPANS treatment, and FPANS also reduced IL-5 and IL-8 production. A comparable reduction was noted in studies by Linden and co-workers using budesonide [15]. Neither drug nor FPANS treatment had any significant effect on the levels of MPO or ENA-78 or on eosinophil numbers. Clearly, modifying adenosine receptor activity does not modulate nasal inflammation to the extent that corticosteroids appear to reduce allergen-induced inflammation in this model.

This study has a number of shortcomings as the drug has dual actions on adenosine receptors A_{2A} and A₃ and, therefore, the response to allergen represents the total of these activities. It is possible that a more pronounced effect would be observed if the compound engaged one and not both receptors and future studies targeting a single receptor will be of interest. The dose of the drug chosen in the current study was limited by the therapeutic index, which is narrow due to side effects, principally tachycardia. It cannot be excluded that higher doses of new compounds with fewer side effects might demonstrate efficacy. FPANS was used as a positive control, which required use of an additional placebo to compensate for the scented smell of this compound (absent in drug). Although comparison of the active medications was

thus made to different placebo compounds on different days, it is unlikely to have biased the results or outcome as all results from the placebo study days were virtually identical. Comparison of the placebo days also confirmed the within-patient repeatability of nasal allergen challenge measurements. Finally, an incomplete block design was chosen to shorten the study period, to make the study less onerous for patients and to reduce the overall costs of the trial. We do not believe that the study was compromised by this strategy, especially given the significant number of subjects who participated in the study. There were no differences in the baseline demographics of the treatment blocks.

In summary, a selective adenosine A_{2A} receptor agonist–A₃ receptor antagonist provided limited benefit with respect to clinical symptoms and nasal airflow measurements. However, release of some mediators (such as tryptase) appeared to be blunted following allergen challenge, suggesting that further studies, using other selective adenosine receptor agonists and antagonists, are needed to understand the role and function of adenosine and to examine therapeutic alternatives in AR.

Acknowledgements

This study was supported by a grant from GlaxoSmith-Kline (GSK). The authors thank all the subjects for their participation in the study and GSK for financial support and assistance with studies. We also thank Andrew Williams (Clinical Immunology, Centenary Institute, New South Wales, Australia) for measuring ECP, tryptase and albumin concentrations in nasal secretions.

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