

Original article

Effect of an inhaled adenosine A_{2A} agonist on the allergen-induced late asthmatic response

Background: Adenosine receptor activation is suggested to play a role in asthmatic airway inflammation. Inhibition of adenosine receptors may have an effect on the late asthmatic response (LAR) after allergen inhalation and this mechanism could offer a potential new treatment in asthma.

Methods: We evaluated the effect of an inhaled adenosine-_{2A} (A_{2A})-receptor agonist (GW328267X), 25 µg, in 15 nonsmoking atopic asthmatics who underwent an inhaled allergen challenge following twice daily treatment for 1 week in a double-blind, placebo- and fluticasone propionate (250 µg) controlled study.

Results: In contrast to fluticasone, treatment with the A_{2A}-receptor agonist neither significantly protect against the allergen-induced early and late asthmatic reaction, nor the accompanying inflammatory response as measured by sputum total cell counts, number of EG2+ cells, and the concentration of interleukin-8 and eosinophil cationic protein.

Conclusion: The inhaled A_{2A}-receptor agonist, GW328267X, 25 µg does not affect the allergen-induced LAR or the associated inflammatory response in asthma.

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Asthma is a chronic inflammatory disorder characterized by recurrent episodes of symptoms of wheezing and chest tightness that are associated with variable airway obstruction and increased airway hyperresponsiveness (1). Several studies have shown a link between adenosine and the pathophysiology of asthma (2–4). It has been found that adenosine levels are elevated in bronchoalveolar lavage fluid and in exhaled breath of asthmatics and these levels increase even further after allergen challenge (4–6). Furthermore, inhalation of adenosine-5'-monophosphate induces airway hyperresponsiveness (3). This raises the possibility that adenosine, once liberated in the airways, can contribute to the pathophysiology of asthma. Adenosine may be involved in asthmatic airway inflammation by acting on four different adenosine receptors, namely A₁, A_{2A}, A_{2B} and A₃ receptors (7, 8). These adenosine receptors have been put forward as a potential target for a new therapeutic approach, as these receptors have been identified on many different inflammatory cells and their activation induces both pro- and anti-inflammatory responses (7).

The A_{2A} receptor is of particular interest, as activation of this receptor induces anti-inflammatory activities, which

opens avenues for treatment of asthma. Several anti-inflammatory actions of adenosine A_{2A} receptors have been described. Activation of T-lymphocytes, which play a key role in the recruitment of leukocytes to the lung, is suppressed by A_{2A}-receptor activation in rats (9). In addition, A_{2A}-receptor activation results in suppression of FcεR1-induced degranulation of human mast cells and the secretion of interleukin-12 (IL-12), a pro-inflammatory cytokine, from human monocytes (10, 11). Activation of A_{2A} receptors also suppresses T-cell effector functions, neutrophil adherence to the endothelium, upregulation of integrins and activation and degranulation of neutrophils (7, 12–16). Furthermore, Ohta and Sitkovsky (17) showed that low doses of an inflammatory stimulus, causing minimal tissue damage in wild-type mice, induced extensive tissue damage, higher levels of pro-inflammatory cytokines and eventually death in animals lacking the A_{2A} receptor. In addition, treatment with an A_{2A}-receptor agonist (CGS 21680) inhibits the late asthmatic response (LAR) in rats (18). Thus, there is ample evidence for an anti-inflammatory effect induced by activation of adenosine A_{2A} receptors. This led us to assess the potential anti-inflammatory effect of an inhaled A_{2A} agonist in asthma.

Recently a new adenosine A_{2A} -agonist GW328267X, has been developed for human use. GW328267X acts as a potent and selective A_{2A} agonist and as a competitive A_3 -antagonist (19). Interestingly, several anti-inflammatory effects of this new adenosine A_{2A} agonist have been found. First, GW328267X inhibits lung eosinophilia and neutrophilia in conscious rats and guinea pigs. In addition, GW328267X inhibits N-formyl-methionyl-leucyl-phenylalanine-induced release of granule proteins from human blood [eosinophilic cationic protein (ECP) and myeloperoxidase]. Finally, GW328267X potently inhibits the generation of reactive oxygen species from human neutrophils and eosinophils. Furthermore, GW328267X partially inhibited the early asthmatic response (EAR) and LAR after nasal allergen challenge in patients with allergic rhinitis (20).

The LAR is supposed to be a representative model to investigate the efficacy of anti-inflammatory drugs (21). We hypothesized that the recently developed adenosine A_{2A} agonist, GW328267X, will have an inhibitory effect on the LAR and associated inflammation in patients with asthma. We therefore, compared the effects of treatment with either the adenosine A_{2A} -agonist GW328267X 25 μ g, fluticasone propionate (FP) 250 μ g, or placebo twice daily for 1 week on an allergen challenge, sputum cell counts, cytokines and toxic proteins in sputum and exhaled nitric oxide (eNO).

Methods

Patients

Fifteen male patients with the following inclusion criteria: a diagnosis of asthma according to the Global Initiative for Asthma guidelines, 18- to 55-year old, nonsmoking, forced expiratory volume in 1 s (FEV_1) $\geq$ 70% predicted, provocative concentration of methacholine inducing a 20% fall in FEV_1 (PC_{20}) histamine $\leq$ 8 mg/ml, and at least one positive skin prick test for house dust mite, cat or timothy grass (22). Subjects who required oral or inhaled corticosteroid treatment during the 8- or 4-week period, respectively, prior to allergen challenge were excluded. In addition, patients were excluded when their asthma was unstable during 4 weeks prior to the allergen challenge or when other medication was necessary with the exception of inhaled short-acting β_2 -agonists, if needed.

Study design

This study was set up as a randomized, double-blind, placebo and inhaled corticosteroid controlled, three-way cross-over study to investigate the effect of treatment with an A_{2A} agonist (GW328267X) on the LAR. Inhaled doses were administered twice daily for 6 days and once on the morning of day 7, using a four-place DiskhalerTM (GlaxoSmithKline, Stevenage, UK). Each actuation contained either 25 μ g of GW328267X, 250 μ g of inhaled fluticasone or placebo. This dose was previously shown to be safe and well tolerated in healthy subjects and in patients with asthma (GlaxoSmithKline data on file and Luijk et al., submitted for publication). On the morning of day 7 the final dose was given 0.5 h prior to the start of an allergen challenge. At the start of each treatment period, all patients had to demonstrate disease stability as defined by a PC_{20} -histamine within

1.5 doubling doses from the individual screening value. Each treatment period was separated by a washout period of 2–3 weeks. Sputum inductions were performed predose on days 4 and 8 24 h after allergen challenge, and eNO was measured predose on days 1, 4 and 7. Blood pressure, heart rate, electrocardiograms and symptoms were recorded to monitor the occurrence of side-effects induced by treatment on days 1, 4 and 7 prior to and after dosing. Blood samples were obtained on days 1 and 7 of each treatment period prior to the dose and up to 6 h after drug inhalation for the assessment of the serum GW328267X concentrations. Blood was also collected for assessment of serum ECP up to 24 h on days 1 and 7. The medical ethical committees of the University Medical Center Utrecht and the University Hospital Groningen approved the study and all patients gave their written informed consent.

Histamine and allergen challenge

We applied the standardized guidelines for histamine and allergen challenge, employing the 2-min tidal breathing method. For the allergen challenge either house dust mite, cat or timothy grass allergen (ALK Abelló, Horsholm, Denmark) was used depending on the individual sensitivity of the subject as determined by skin prick test at screening (23, 24).

Sputum induction and processing for the assessment of sputum inflammatory markers

Sputum was induced by inhalation of hypertonic saline aerosols and processed as previously described (25–28). Sputum cell counts and concentrations of ECP, IL-8 in sputum fluid phase, EG2 positive sputum cells and ECP in the blood were measured (ImmunoCAP ECP; Pharmacia, Uppsala, Sweden). The concentration of IL-8 in sputum fluid phase was measured by ELISA (CLB, Amsterdam, The Netherlands). Immunohistochemical staining was performed on sputum cytopins by an antibody clone EG2 that binds to human eosinophil cationic protein (Kabi Pharmacia Diagnostics, Uppsala, Sweden) to determine the percentage of positive cells.

Exhaled nitric oxide

Exhaled NO was measured according to the American Thoracic Society standards at the same time of day for all treatment periods and was expressed as parts per billion (29).

Statistical analysis

All data were analysed using an intention-to-treat analysis. The $EAR_{(0-2\text{ h})}$ and $LAR_{(3-8\text{ h})}$ expressed as the decline in FEV_1 in litres (l) were assessed by analysing the weighted mean as derived from the area under the curve divided by the time interval [$AUC_{(EAR: 0-2\text{ h})}$ and $AUC_{(LAR: 3-8\text{ h})}$ postallergen challenge, respectively] and the lowest (minimum) FEV_1 during EAR and LAR using ANCOVA. A fixed effects model was employed with factors for period, treatment and subject, with baseline FEV_1 at day 1 as the covariate. Differences between each treatment (GW328267X or FP) and placebo were calculated along with the associated 95% CI between brackets. Following a natural logarithmic transformation, sputum inflammatory cells and inflammatory markers in sputum fluid phase (IL-8 and ECP), EG2 positive cells in sputum, serum ECP and eNO were analysed using ANCOVA with factors for period, treatment, time, treatment by time interaction, subject and predose day 1 values as a covariate (with the exception of induced sputum measurements). Differences between each treatment were expressed in

terms of the ratio of the least squares geometric means and presented along with the associated 95% CI (ratio: GW328267X/placebo and FP/placebo). A ratio of 1 thus indicates no effect of GW328267X or FP compared with placebo.

Results

Fifteen patients were included and 14 patients completed the study; baseline characteristics are presented in Table 1. Fourteen patients showed stable PC₂₀ histamine prior to start of each treatment period (data on PC₂₀ histamine are not shown). One patient complained of shortness of breath on the third day of dosing with the active drug (GW328267X), although there was also a natural exposure to cats at home during this treatment period. This side-effect was graded as mild and lasted 8 days after the final dose and was a reason to discontinue treatment. Other than this isolated event all treatments were generally well-tolerated. The pharmacokinetic profile showed a rapid increase of GW328267X in the peripheral blood with a peak within 0.5 h [t_{max} : 0.42 (0.25–0.75) h on day 1 and 0.25 (0.08–1.00) h on day 7].

Effects on FEV₁ during the EAR and LAR

Treatment with the A_{2A} agonist (GW328267X) did not protect against the EAR or the LAR (LAR: minimum FEV₁: –0.20 (95% CI: –0.57 to 0.16) L and weighted mean FEV₁: –0.144 (–0.44 to 0.15) L) when compared with placebo, whereas a significant protective effect was demonstrated against both the EAR [minimum FEV₁: 0.86 (0.55–1.16) l and weighted mean FEV₁: 0.53 (0.30–0.76) l] and the LAR [minimum FEV₁: 1.19 (0.85–1.54) l and weighted mean FEV₁: 0.85 (0.58–1.13) l] after FP treatment when compared with placebo (Fig. 1).

Effect on sputum cell differential counts

In Table 2, the total and cell differential counts are listed. Treatment with the A_{2A} agonist (GW328267X) did not

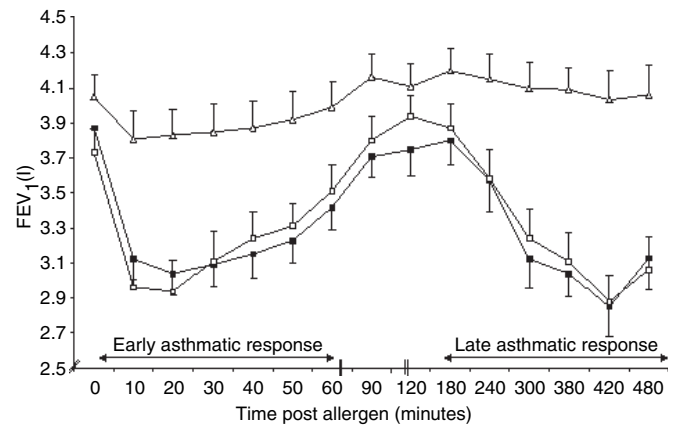


Figure 1. Decrease in mean FEV₁ (l) (with the associated SE of the mean) after allergen challenge on day 7 of treatment with either placebo (open squares), an A_{2A}-agonist GW328267X (solid squares), or fluticasone propionate (FP) (open triangles) inhalations twice daily during the early and the late asthmatic response (LAR). No significant difference in effects between the A_{2A} agonist (GW328267X) and placebo was observed, whereas FP induced a significant inhibition of both the early and the LAR.

significantly change sputum total or cell differential counts after 3 days of treatment (predose on day 4) or 24 h after allergen challenge (day 8) when compared to placebo. Although treatment with FP did not significantly decrease the percentage of sputum eosinophils on day 4 [ratio: 0.391 (95% CI: 0.120–1.270); Fig. 2A], there was a significant inhibition of the increase in sputum eosinophils 24 h after allergen challenge [ratio: 0.291 (95% CI: 0.089–0.956); Fig. 2B]. In addition, no difference was found in sputum neutrophils on day 4 of FP treatment, although the percentage of sputum neutrophils significantly increased 24 h after allergen challenge (day 8) during treatment with FP when compared with placebo treatment [ratio: 3.135 (1.165–8.434)]. The other sputum cell differential counts on day 4 and 24 h after allergen challenge (day 8) during treatment with either the A_{2A} agonist (GW328267X) or FP were not significantly different from placebo.

Effect on soluble inflammatory markers in sputum and blood

In Table 2 the soluble sputum inflammatory markers are listed. No differences in the ratio of concentrations of sputum ECP, IL-8 and the number of EG2 positive cells were found between treatment with the A_{2A} agonist and placebo after 3 days treatment (predose on day 4) or 24 h after allergen challenge (day 8). In contrast, FP decreased the concentration of sputum ECP when compared to placebo 24 h after allergen challenge, whereas this effect was not significant on day 4 [ratios 0.480 (95% CI: 0.217, 1.058 on day 4, and 0.415 (95% CI: 0.188, 0.917) on day 8]. No differences were seen in blood ECP concentrations after treatment with the A_{2A} agonist (GW328267X) or FP when compared with placebo (data not shown).

Table 1. Patient characteristics

	Mean ± SD
n	15
Age (years)	28 ± 12
FEV ₁ (% predicted)	95 ± 13
PC ₂₀ histamine (mg/ml)*	0.86 (0.67–2.3)
Allergen†	10 H; 4 G; 1 C
EAR _(0-2 h) : % decline in FEV ₁ from baseline	22 ± 4.1
LAR _(3-8 h) : % decline in FEV ₁ from baseline	30 ± 12

FEV₁, forced expiratory volume in 1 s; PC₂₀, provocative concentration of methacholine inducing a 20% fall in FEV₁; EAR, early asthmatic response; LAR, late asthmatic response.

*Geometric mean (95% CI).

†Allergens used during challenges: H, house dust mite; G, grass; C, cat.

Table 2. sputum cell counts (%), ECP and IL-8 in the sputum fluid phase and EG2 positive cells on day 4 during treatment and 24 h after allergen challenge on day 8

	Day 4			Day 8		
	A _{2A} agonist	Placebo	FP	A _{2A} agonist	Placebo	FP
Total cells (×10 ⁶ /ml)	3.71 (2.03–6.80)	3.44 (1.66–7.11)	4.61 (2.53–8.39)	7.29 (4.08–13.03)	4.37 (2.44–7.82)	5.40 (2.52–11.57)
Macrophages	42.86 (2.41–56.68)	45.51 (31.41–65.92)	52.15 (35.88–75.79)	29.34 (19.82–43.43)	28.75 (17.91–46.16)	13.52 (3.20–57.14)
Lymphocytes	1.32 (0.61–2.89)	1.97 (0.70–5.58)	0.92 (0.32–2.61)	1.12 (0.31–4.04)	1.44 (0.51–4.08)	2.11 (0.71–6.27)
Neutrophils	23.54 (12.83–43.19)	15.22 (5.29–43.77)	14.18 (4.34–46.31)	15.46 (5.37–44.50)	11.76 (2.65–52.26)	35.46* (21.42–58.70)
Eosinophils	4.73 (1.55–14.43)	4.55 (1.51–13.74)	1.69 (0.51–5.55)	27.28 (20.10–37.02)	14.43 (4.75–42.76)	4.36* (1.38–13.79)
ECP (ng/l)	64.66 (32.63–128.11)	74.11 (23.06–238.22)	39.90 (20.84–76.41)	153.59 (50.29–469.09)	106.35 (30.86–366.56)	49.59* (18.59–132.29)
IL-8 (pg/l)	3867 (501–5978)	5058 (3249–7875)	3669 (2207–6099)	4340 (2372–7940)	3143 (1134–8710)	4151 (2237–7702)
EG2+ (%)	2.96 (0.62–14.10)	2.40 (0.47–12.30)	0.88 (0.13–5.93)	7.04 (1.51–32.78)	6.21 (1.14–33.97)	4.73 (1.18–19.04)

Data expressed by geometric means (95% CI) for the adenosine _{2A}-receptor agonist (GW328267X), placebo and FP.
FP, fluticasone propionate; ECP, eosinophilic cationic protein.
*Statistically different from placebo (see text for treatment difference).

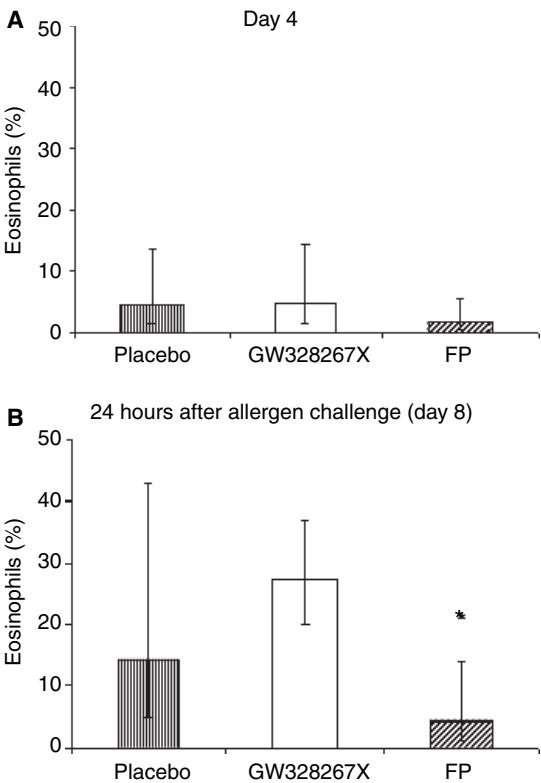


Figure 2. (A) Percentage of sputum eosinophils predose on day 4 of treatment with either the adenosine A_{2A} agonist (GW328267X), fluticasone propionate (FP), or placebo treatment (geometric means and 95% CI). (B) Percentage of sputum eosinophils 24 h after allergen challenge (day 8) after either the adenosine A_{2A} agonist (GW328267X), FP, or placebo (geometric means and 95% CI). FP treatment significantly inhibited the increase in the percentage of eosinophils after allergen challenge compared to placebo (marked by *, for details see text), whereas no difference was found between GW328267X and placebo.

Effect on eNO measurements

The mean values of the eNO concentrations are shown in Fig. 3. There were no significant differences in the

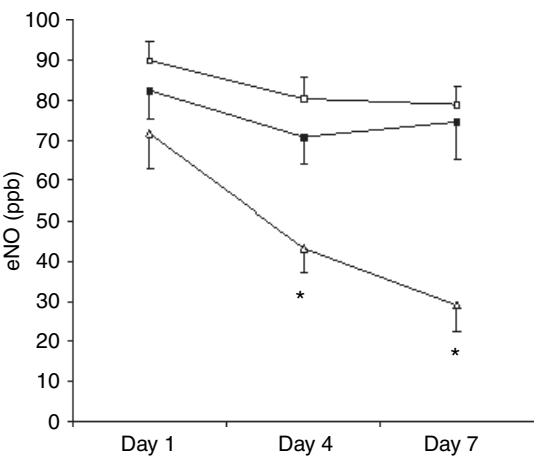


Figure 3. The mean (and SE of the mean) of exhaled nitric oxide concentration in parts per billion (ppb) during treatment with either an inhaled A_{2A} agonist GW328267X (solid squares), placebo (open squares) or fluticasone propionate (FP) (open triangles) predose on days 1, 4 and 7. A significant decrease was observed after FP treatment on days 4 and 7 [*significant difference compared with placebo (see text)].

ratio of eNO concentrations between the A_{2A} agonist (GW328267X) and placebo on days 4 and 7 of treatment. However, there were significant decreases in the concentrations of eNO at the corresponding time points for FP compared with placebo [ratio on day 4: 0.56 (0.46–0.67) and ratio on day 7: 0.41 (0.34–0.49)].

Discussion

In this study, we found that treatment with the inhaled adenosine A_{2A} agonist, (GW328267X, 25 µg twice daily) did not protect against the LAR, expressed as the decline in FEV₁ 5–8 h after allergen challenge, or the accompanying increase in airway inflammation in asthmatic subjects, whereas FP did so as a positive control. In addition, we did not find a reduction in airway

inflammation after treatment with GW328267X as expressed as cell differential and inflammatory markers in sputum and blood.

Our results are not in agreement with the findings of Rimmer et al. (20) using the same drug GW328267X in allergic rhinitis patients. They found limited effects on the EAR and LAR after nasal allergen challenge and clinical symptoms and a small effect on reduced tryptase nasal lavage levels, but no effect on other mediators. We, however, did not measure tryptase. In addition, a recent study found that after an acute ovalbumin challenge of sensitized mice, administration of a selective adenosine A_{2A} agonist CGS21680 inhibited the bronchoalveolar lavage fluid inflammatory cell influx but not the airway hyperresponsiveness and the allergen-induced bronchoconstriction (30). In contrast, Fozard et al. (18) found that treatment with a selective A_{2A} agonist (CGS21680) significantly inhibited the allergen-induced LAR in rats. There are several possible explanations for the observed discrepancies. First, the A_{2A} agonist we used (GW328267X) was not entirely selective for the A_{2A} receptor, but also exhibited some inhibitory effect on the A₃ receptor. It has been suggested that inhibition of the adenosine A₃ receptor may have anti- or pro-inflammatory effects, as it was found that activation of the adenosine A₃ receptor could either reduce eosinophil chemotaxis and neutrophil degranulation induced by lipopolysaccharide (LPS) or TNF- α or instead, induce hypersecretion of mucus and migration of eosinophils to the airways (31). Thus, the inhibitory effect of GW328267X on A₃ receptors may have counteracted possible beneficial effects of A_{2A}-receptor activation. Secondly, adenosine receptor subtypes involved in adenosine-induced bronchoconstriction and inflammatory responses vary among species and this makes the clinical relevance of data obtained in animal models unclear. In addition, the specific tissue and cell type distribution of adenosine receptors in human upper and lower airways is not clear (32, 33). Thirdly, it could be argued that the dose of the A_{2A} agonist we used (25 μ g, twice daily) was subtherapeutic either because of a too low dose or too short duration of therapy. The dose of 25 μ g GW328267X twice daily was chosen, as it was an intended clinical dose and had previously shown to be safe and well tolerated in healthy subjects and patients with asthma (GSK data on file), whereas higher doses of GW328267X are accompanied by cardiovascular effects (decrease in blood pressure and an increase in heart rate; GSK data on file). It remains possible that we may have found beneficial effects, if we would have been able to administer higher doses of GW328267X.

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Nevertheless, we have chosen to investigate the effect of the A_{2A}-agonist GW328267X on the inhaled allergen response and we have analyzed 15 patients for this purpose. We feel confident that the sample size was appropriate, as it has been shown that inclusion of 12 patients is already adequate to reliably demonstrate 50% attenuation of the LAR with >90% power (21). In agreement with this, we were able to demonstrate a significant attenuation of the LAR after 1 week of treatment with inhaled fluticasone 250 μ g twice daily. The question remains whether a lack of any effect on the allergen-induced late bronchoconstrictor or inflammatory response preclude an effective anti-allergic or anti-inflammatory drug. We are unaware of any drug making it into clinical medicine, when failing to attenuate the LAR, and we therefore think it is unlikely that A_{2A}-receptor agonism by GW328267X at a dose of 25 μ g twice daily will prove to be an effective asthma therapy. In this context, it would be interesting to investigate a possible synergistic effect by combining an A_{2A}-agonist with an inhaled corticosteroid in future studies.

Finally, a surprising finding in this study was that the percentage of sputum neutrophils after allergen challenge was higher after 1 week treatment with inhaled fluticasone when compared with placebo. The increase in neutrophils after fluticasone may be explained by reduced apoptosis or alternatively by enhanced recruitment of neutrophils to the airways after allergen challenge as we found no increase in sputum neutrophils after 4 days treatment prior to allergen challenge (34). This increase in neutrophils may also be caused by upregulating endogenous synthesis of leukotriene B₄ by corticosteroids and presence of IL-8 (35). However, we did not find elevated sputum levels of IL-8 and the relevance of these elevated sputum neutrophils has to be further investigated.

In conclusion, we have investigated the clinical consequences of activation of the adenosine A_{2A} receptor and we could not demonstrate a beneficial effect on the EAR, and the LAR, or the associated increase in airway inflammation. Despite these results with the inhaled A_{2A}/A₃-antagonist GW328267X at a dose of 25 μ g twice daily for 1 week, we feel that the role of adenosine and adenosine receptors in asthma deserves further study. It still remains possible that modulation of adenosine receptor subtypes will have therapeutic implications in asthma.

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