Effect of Wortmannin on Human Eosinophil Responses *in Vitro* and on Bronchial Inflammation and Airway Hyperresponsiveness in Guinea Pigs *in Vivo*

CHARLES I. EZEAMUZIE, JALAJA SUKUMARAN, and ELIZABETH PHILIPS

Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, Safat, Kuwait

Many mediators activate eosinophils via transduction pathways involving the enzyme phosphatidylinositol 3-kinase. The initial investigation of wortmannin, a specific inhibitor of PI3-kinase, was of its effect on human and guinea pig eosinophil superoxide (O_2^-) release and degranulation *in vitro*. Subsequently, the effect on allergen- and Sephadex-induced bronchial inflammation and airway hyperresponsiveness (AHR) *in vivo* in guinea pigs was investigated. Wortmannin potently inhibited complement C5a-induced O_2^- generation and eosinophil peroxidase (EPO) release from human eosinophils, with 50% inhibition produced by a 1–10 nM concentration. Both aerosol allergen challenge of sensitized guinea pigs and intravenous injection of Sephadex beads in normal guinea pigs caused, in 24 h, significant eosinophilia and increased EPO activity in bronchoalveolar lavage fluid (BALF) and AHR to intravenous acetylcholine and histamine. In the allergic model, intranasal pretreatment with wortmannin had no effect on BALF eosinophilia, but dose dependently inhibited BALF EPO activity. At 1 mg/kg, the drug abolished the AHR to histamine, but not acetylcholine. In the Sephadex model, the drug significantly inhibited all three parameters (eosinophilia, increased EPO activity, and AHR to both spasmogens). These results show that wortmannin is a potent inhibitor of human eosinophil degranulation and that when administered intranasally can prevent AHR in allergen-challenged guinea pigs, probably by inhibiting eosinophil degranulation, but not their accumulation in BALF. This may be relevant to the possible clinical utility of wortmannin in conditions involving eosinophilic inflammation and AHR.

Keywords: airway hyperresponsiveness; eosinophils; guinea pigs; wortmannin

Lung inflammation and airway hyperresponsiveness (AHR) are important components of bronchial asthma (1, 2), and several reports have shown that eosinophil accumulation and subsequent activation in bronchial tissues play a critical role in the pathophysiology of the disease (3, 4).

Although the exact causal relationship between bronchial eosinophilic inflammation and AHR is not clearly understood, it is currently believed that the eosinophils infiltrating the asthmatic lung degranulate to release tissue-damaging granular proteins such as the major basic protein and eosinophil peroxidase (EPO), as well as release oxygen free radicals. These mediators, acting in concert, may then cause airway epithelial damage, resulting in the development of AHR (5, 6).

Wortmannin is a relatively specific inhibitor of phosphatidylinositol 3-kinase (PI 3-kinase) (7). The use of this inhibitor has revealed the involvement of PI 3-kinase in the biochemical transduction of activation signals generated by many inflammatory mediators in eosinophils. For example, in isolated human eosinophils, wortmannin potently blocks neuropeptide-induced chemotaxis (8) and interleukin 5 (IL-5)-induced β_2-integrin-dependent adhesion to intercellular adhesion molecule type 1 (ICAM-1)-coated surfaces (9). It also inhibited C5a-induced generation of leukotrienes (10), phosphoinositide metabolism, and production of reactive oxygen metabolites (11, 12). In guinea pigs, Palframan and coworkers (13) showed that wortmannin inhibited IL-5-induced selective release of eosinophils from perfused bone marrow, as well as selective eosinophil chemokinesis *in vitro*. These observations may suggest a potential effect for wortmannin in preventing the development of AHR *in vivo*, either by preventing the eosinophil infiltration of bronchial tissues or their activation on arrival. Despite the studies described above, none, to our knowledge, have examined the effect of this drug *in vivo* in allergen-induced eosinophilic lung inflammation and/or AHR in any animal models. One reason may be that wortmannin, being an inhibitor of a widely distributed enzyme, may have an effect too unspecific to be useful.

In the present study, we set out first to confirm that wortmannin inhibits eosinophil responses *in vitro* and subsequently to study its effect on bronchial inflammation and AHR. To do this, we employed two guinea pig models, the allergen- and Sephadex-induced models of bronchial inflammation/AHR, and administered the drug by intranasal instillation to localize the effect to the lungs. The study also allowed us to assess the relative role of airway eosinophilia and eosinophil degranulation in the development of AHR in guinea pigs.

METHODS

Isolation of Human Blood Eosinophils

Granulocytes were isolated from citrate-anticoagulated (13 mM final concentration) blood (14) from consenting healthy adults according to a protocol approved by the Faculty of Medicine Ethics Committee (Kuwait University, Safat, Kuwait). Eosinophils were purified from the granulocyte fraction by the immunomagnetic method (15), using mouse anti-human CD16 monoclonal antibody to positively extract neutrophils. Eosinophils were 98% pure and greater than 98% viable.

Allergen- and Sephadex-induced Lung Inflammation

Male Dunkin Hartley guinea pigs (300–500 g) were sensitized by intraperitoneal injection of 10 μg of ovalbumin (OA) and 5 mg of $Al(OH)_3$ in 1 ml of sterile saline on Days 1 and 7. Controls received $Al(OH)_3$ alone. Twenty-one to 28 d later, all animals were pretreated with pyrilamine maleate (10 mg/kg, intraperitoneal; 1 h prior), and challenged with aerosolized OA (0.5% [w/v] in saline) or saline for 30 min.

For the Sephadex model, lung inflammation, was induced by a single intravenous injection, via the foot vein under local anesthetic (2% xylocaine solution) and light ether anesthesia, of a sterile suspension of Sephadex G-50 beads (24 mg/kg in saline) as previously described (16). Inflammation and hyperreactivity were assessed 24 h later.

Bronchoalveolar Lavage, Cell Count, and Isolation of Guinea Pig Bronchial Eosinophils

Animals were killed with an overdose of pentobarbitone sodium and bronchoalveolar lavage was performed as previously reported (17). The

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Correspondence and requests for reprints should be addressed to Charles I. Ezeamuzie, Ph.D., Department of Pharmacology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait. E-mail: ezeamuzie@hsc.kuniv.edu.kw

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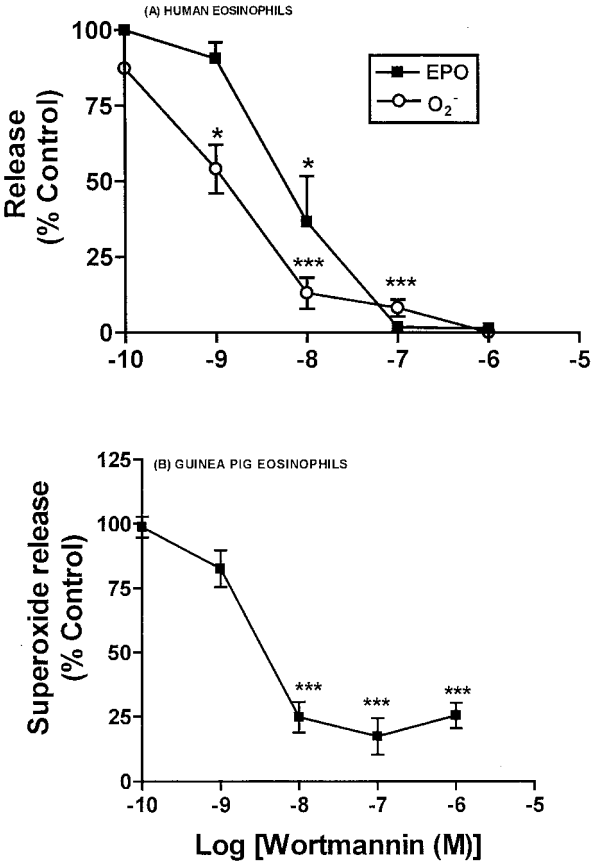

Figure 1. Inhibition by wortmannin of human and guinea pig eosinophil responses *in vitro*. (*A*) inhibition of O_2^- and EPO releases from human blood eosinophils stimulated with C5a (10^{-8} M). (*B*) Inhibition of PAF-induced O_2^- release from guinea pig eosinophils. The net uninhibited releases were as follows: 15–34% of total cell EPO content and 18–40 nmol of cytochrome *c* reduced per 10^6 cells per h for human and guinea pig eosinophil O_2^- release. All values represent means ± SEM, n = 5–8. *p < 0.05; ***p < 0.001.

cells were separated and the supernatant was saved for determination of EPO activity. Total and differential counts were performed by using a standard hemocytometer and Wright–Giemsa-stained cytosmear.

Guinea pig eosinophils were isolated from normal animals and purified by centrifugation over a Percoll gradient, and eosinophils (92–98% purity) were recovered as the pellet. The purified cells were then washed twice, counted, and then used for *in vitro* superoxide anion (O_2^-) release experiments.

In Vitro Release of Superoxide Anions and Eosinophil Peroxidase

Superoxide anion generation from eosinophils was determined by the superoxide dismutase-inhibitable reduction of ferricytochrome *c* (18). Purified cells were incubated with wortmannin for 10 min, stimulated with C5a (human) or platelet-activating factor (PAF, guinea pig), and incubated at 37° C for 1 h, and the absorbance was read at 550 nm. The amount of O_2^- generated was estimated as nanomoles of ferricytochrome *c* reduced per 10^6 cells per hour, using the extinction coefficient of 2.1×10^{-4}/M/cm.

For EPO release, human eosinophils were incubated with cytochalasin B (5 μg/ml) for 5 min, and then stimulated with C5a for 30 min. Cells were removed and EPO was measured in the supernatant, as well as in Triton X-100–lysed cells.

EPO activity was measured by the *o*-phenylenediamine method as previously reported (19). For EPO released from human eosinophils *in vitro*, values were expressed as a percentage of total cell content, using the amount obtained in half the same number of cells, after lysis, as 50%. The amount of EPO in guinea pig bronchoalveolar lavage fluid (BALF) was determined from a standard curve constructed with horseradish peroxidase (Type 1, 0.1–15 ng/ml; Sigma, St. Louis, MO).

Treatment Groups and Drug Administration

For the assessment of AHR and BALF EPO activity, the data from saline/saline (SAL/SAL) and OA/SAL groups were combined into a control group (CONTR), as there were no significant differences between them with regard to these two parameters.

Wortmannin (dissolved in 10% dimethyl sulfoxide [DMSO, in saline]) or vehicle was administered in a volume of 200 μl by intranasal instillation to each animal 1 h before and 3 h after allergen challenge or Sephadex injection.

Measurement of Lung Function

Guinea pigs were anesthetized with urethane (175 mg/kg, intraperitoneal) and paralyzed with pancuroniun bromide (50 μg/kg, intravenous). They were ventilated with room air (8 ml/kg, 1 Hz) using a model 683 ventilator (Harvard Apparatus, Natick, MA). Air flow and transpulmonary pressure were measured as previously reported (20). Lung resistance (R_L) and dynamic compliance (Cdyn) were calculated by a digital lung function recording system (Mumed Systems, London, UK).

To assess airway hyperresponsiveness, acetylcholine (ACh) and histamine, both in the range of 1–128 μg/kg, were administered intravenously at 5-min intervals via a cannulated femoral vein. In experiments in which both spasmogens were tested, ACh was used first.

Drugs and Chemical Reagents

Recombinant human C5a, wortmannin, *o*-phenylenediamine, PAF, histamine dihydrochloride, ACh, urethane, Sephadex G-50, Percoll, and cytochalasin B were obtained from Sigma. Mouse monoclonal anti-human CD16 antibody (clone FcR gran1) was obtained from

TABLE 1. EFFECT OF WORTMANNIN ON TOTAL AND DIFFERENTIAL CELL COUNTS IN BALF RECOVERED FROM SENSITIZED GUINEA PIGS 24 h AFTER AEROSOL ALLERGEN CHALLENGE

Sensitization/ Challenge	Drug Treatment	N	Total cells ($\times 10^6$)	Eosinophils ($\times 10^6$)	Eosinophils (%)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)
SAL/SAL	SAL	12	45.83 ± 6.42	6.25 ± 0.80	13.9 ± 1.8	79.3 ± 2.2	5.0 ± 0.7	0.4 ± 0.2
SAL/SAL	VEH	5	64.20 ± 15.72	12.20 ± 4.61*	19.0 ± 6.8	72.8 ± 8.9	4.5 ± 0.6	1.3 ± 0.3*
SAL/SAL	WTM (1 mg)	3	47.00 ± 10.22	14.30 ± 0.84*	30.4 ± 1.8*	62.8 ± 3.9	4.7 ± 0.7	2.1 ± 0.6*
OA/SAL	VEH	8	53.85 ± 15.81	12.78 ± 0.49	23.8 ± 1.0	56.0 ± 5.0	11.6 ± 1.8	1.1 ± 0.5
OA/OA	SAL	13	136.60 ± 11.30†	54.52 ± 4.76†	40.0 ± 3.5*	52.2 ± 3.4	5.9 ± 0.5	2.4 ± 0.4
OA/OA	VEH	13	110.53 ± 11.30‡	48.62 ± 3.10†	44.0 ± 2.9‡	48.1 ± 3.1	5.9 ± 1.2	2.9 ± 0.3
OA/OA	WTM (0.3 mg)	4	102.74 ± 32.65	52.20 ± 3.77	50.8 ± 3.7	45.0 ± 3.6	3.5 ± 1.3	1.7 ± 0.3
OA/OA	WTM (0.7 mg)	4	120.72 ± 25.61	49.19 ± 2.27	40.8 ± 1.9	53.0 ± 1.8	5.1 ± 0.7	1.6 ± 0.2
OA/OA	WTM (1 mg)	12	111.90 ± 13.53	46.31 ± 2.30	41.4 ± 2.1	51.3 ± 2.8	6.1 ± 1.2	1.8 ± 0.3

Definition of abbreviations: OA = ovalbumin; SAL = saline; VEH = vehicle; WTM = wortmannin.
* $p < 0.05$, compared with SAL/SAL-SAL.
† $p < 0.001$, compared with OA/SAL-VEH.
‡ $p < 0.01$, compared with OA/SAL-VEH.

TABLE 2. EFFECT OF WORTMANNIN ON TOTAL AND DIFFERENTIAL CELL COUNTS IN BALF RECOVERED FROM NORMAL GUINEA PIGS 24 h AFTER INTRAVENOUS INJECTION WITH SEPHADEX

Induction	Drug Treatment	N	Total cells ($\times 10^6$)	Eosinophils ($\times 10^6$)	Eosinophils (%)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)
Saline	VEH	4	52.62 ± 6.80	7.79 ± 2.10	14.8 ± 3.8	80.6 ± 5.3	7.0 ± 2.8	0.5 ± 0.3
Sephadex	SAL	5	80.13 ± 18.62	27.38 ± 3.47*	34.2 ± 4.3†	51.2 ± 3.3	8.2 ± 1.6	2.2 ± 0.2
	VEH	5	115.70 ± 15.13*	49.69 ± 4.07‡	48.4 ± 4.0‡	43.1 ± 4.5*	4.6 ± 1.2	1.6 ± 0.2
	WTM (1 mg)	5	46.7 ± 16.44§	20.39 ± 2.21‖	43.7 ± 4.7	46.9 ± 3.9	7.5 ± 1.4	2.6 ± 0.2

Definition of abbreviations: SAL = saline; VEH = vehicle; WTM = wortmannin.
* $p < 0.01$, compared with saline plus vehicle.
† $p < 0.05$, compared with saline plus vehicle.
‡ $p < 0.001$, compared with saline plus vehicle.
§ $p < 0.05$, compared with Sephadex plus vehicle.
‖ $p < 0.001$, compared with Sephadex plus vehicle.

CLB (Amsterdam, The Netherlands). Immunomagnetic beads were supplied by Dynal (Oslo, Norway).

Statistics

Experimental data are presented as means ± SEM. For *in vitro* experiments, statistical significance was determined by one-sample *t* test (InStat; GraphPad Software, San Diego, CA). For the *in vivo* experiments, treatment groups were compared by one-way analysis of variance (ANOVA), and when significance was indicated, the Bonferroni test was applied. In all cases, significance was set at $p < 0.05$.

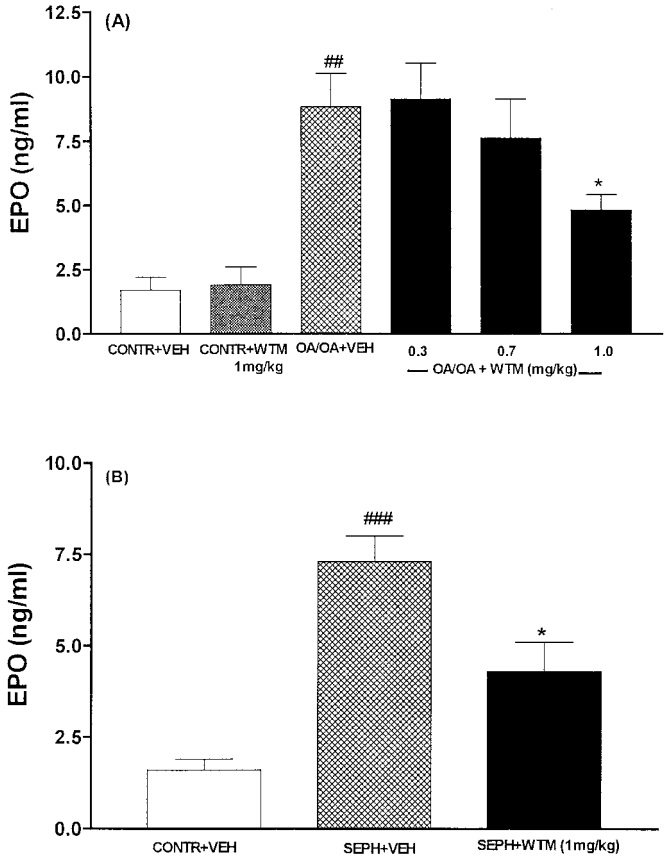

Figure 2. Effect of intranasally administered wortmannin on the level of EPO content of BALF supernatant, 24 h after induction of bronchial inflammation in guinea pigs. (*A*) Effect of pretreatment with wortmannin (WTM) or drug vehicle (VEH) in control animals (CONTR) and in ovalbumin-sensitized and challenged guinea pigs (OA/OA). (*B*) Effect of wortmannin (1 mg/kg) in animals injected with Sephadex beads (SEPH). Values represent means ± SEM, n = 5–8 per group. *$p < 0.05$ compared with OA/OA+VEH or SEPH+VEH, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared with CONTR+WTM and CONTR+VEH, respectively.

RESULTS

Effect of Wortmannin on Human and Guinea Pig Eosinophil Responses *in Vitro*

As shown in Figure 1A, preincubation of human eosinophils with wortmannin caused a concentration-dependent inhibition of O_2^- release induced by 10^{-8} M C5a. In the presence of cytochalasin B (5 μg/ml), C5a induced EPO release from the eosinophils, which was similarly inhibited by wortmannin. The concentration of the drug producing 50% inhibition of response (IC_{50}) (95% confidence interval, 95% CI) was 2.4 (1.2–4.5) nM for O_2^- release and 7.8 (3.6–11.8) nM for EPO release. In the same concentration range, the drug also inhibited PAF-induced O_2^- release from guinea pig bronchial eosinophils, with an IC_{50} (95% CI) of 6.5 nM (3.8–9.2 nM) (Figure 1B). The effect of wortmannin on EPO release from guinea pig eosinophils was not determined because of the lack of an appropriate inducer for this response.

Effect of Wortmannin on Allergen- and Sephadex-induced Cell Content of BALF

As shown in Table 1, the "saline-sensitized" and challenged control group that had been treated with intranasal saline 24 h before bronchial lavage (SAL/SAL+SAL) had a baseline total cell count of 45.83 (±6.42) × 10^6 cells, of which 13.9% were eosinophils. Intranasal instillation of such animals with the drug vehicle alone (SAL/SAL+VEH) had no significant effect on the total cell count, but significantly increased the number of eosinophils (6.25 [±0.80] versus 12.2 [±4.61] × 10^6 cells, $p < 0.05$) and percentage neutrophils (0.4 [±0.2] versus 1.3 [±0.3]%, $p < 0.05$). No further significant change was seen when such animals were treated with wortmannin at 1 mg/kg (SAL/SAL+WTM). In OA-sensitized and challenged guinea pigs that had been pretreated with the drug vehicle (OA/OA+VEH), there was a highly significant increase in the number of cells recovered from BALF compared with the appropriate control group (OA/SAL+VEH) (110.53 [±11.30] versus 53.85 [±15.81] × 10^6 cells, $p < 0.01$). This increase was almost exclusively due to increase in eosinophil count (48.62 [±3.10] versus 12.78 [±0.49] × 10^6 cells, $p < 0.001$). Pretreatment of such animals with wortmannin (0.3–1 mg/kg, intranasal), produced no significant effect on BALF eosinophilia. The drug vehicle alone had no significant effect on any of these parameters in allergen-sensitized and challenged animals.

Similarly, 24 h after intravenous administration of Sephadex into naive guinea pigs, there was a significant increase in the total cells recovered from BALF (115.70 [±15.13] × 10^6 cells in Sephadex-injected guinea pigs, compared with 52.62 [±6.80] × 10^6 cells in saline-injected guinea pigs, $p < 0.01$, both pretreated with drug vehicle) (Table 2). As seen in the allergen model, most of the increase was in the eosinophil

count. In striking contrast to the allergen model, pretreatment with wortmannin (1 mg/kg) completely abolished the increase in total cell number, although no changes were apparent in the percentage composition of the different cells.

Effect of Wortmannin on BALF EPO Activity of Allergen- and Sephadex-induced Lung Inflammation

The amount of EPO in the BALF supernatant of allergen-sensitized and challenged guinea pigs was significantly increased when compared with the controls, both pretreated with drug vehicle (8.8 ± 1.3 ng/ml [n = 8] versus 1.7 ± 0.5 ng/ml [n = 7]; $p < 0.01$) (Figure 2A). Pretreatment with wortmannin (0.3–1 mg/kg, intranasal) caused a dose-dependent decrease in the EPO content of BALF, with the 1 mg/kg dose producing a statistically significant inhibition of 45.5%, $p < 0.05$.

Sephadex injection also caused a significant increase in BALF supernatant EPO after 24 h, when compared with the saline-injected group (7.3 ± 0.7 ng/ml [n = 6], versus 1.6 ± 0.3 ng/ml [n = 8]; $p < 0.001$), (Figure 2B). Pretreatment with wortmannin (1 mg/kg) significantly inhibited the increase by 41.1%, $p < 0.05$.

Effect of Wortmannin on Allergen- and Sephadex-induced AHR

Twenty-four hours after a single aerosol allergen challenge of sensitized guinea pigs, significant AHR to both ACh and histamine was seen (Figure 3A and 3B). This was characterized mainly as an increase in the slope of the dose–response curve of the spasmogens. For example, at 64 μg of ACh per kg, the increase in R_L was 401.7 ± 108.0 cm H_2O/L per s (n = 6) in vehicle-pretreated controls compared with 824.0 ± 122.5 cm H_2O/L per s (n = 6) in sensitized and allergen-challenged animals, also pretreated with the drug vehicle, $p < 0.05$ (Figure 3A). For histamine (32 μg/kg), the corresponding values were 370.3 ± 104.0 cm H_2O/L per s (n = 5), and 692.2 ± 132.3 cm H_2O/L per s (n = 5), $p < 0.05$ (Figure 3B). For both spasmogens, there was little or no increase in the sensitivity. The pretreatment of allergen-sensitized and challenged animals with wortmannin (1 mg/kg, intranasal), a dose that had been established to be effective in inhibiting BALF EPO release, produced only a small, statistically nonsignificant attenuation of the allergen-induced AHR to ACh (Figure 3A). In contrast,

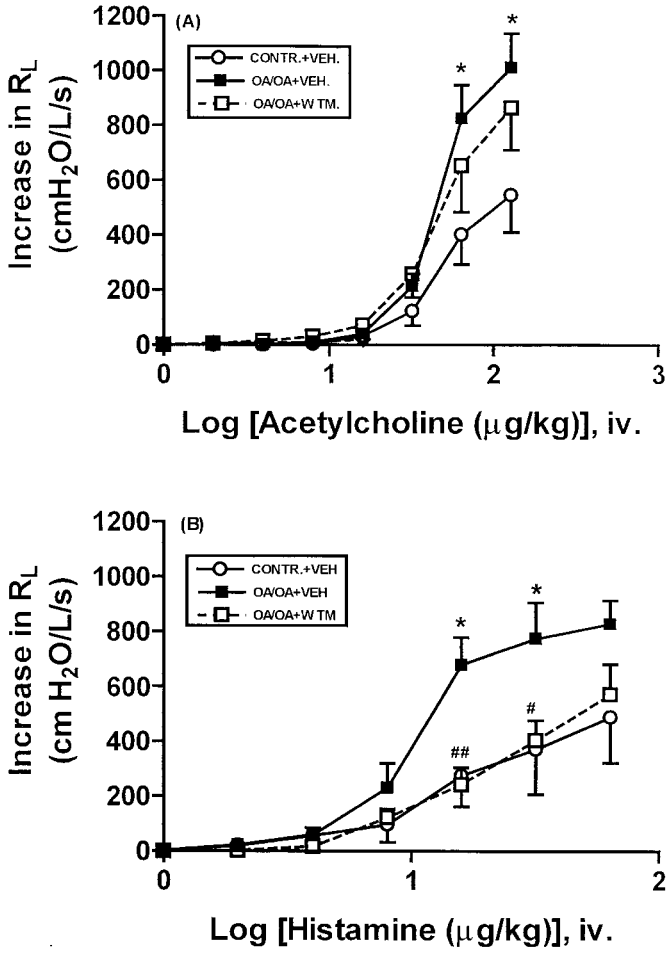

Figure 3. Effect of intranasally administered wortmannin (WTM) or 10% DMSO vehicle (VEH) on the development of AHR, 24 h after allergen challenge of sensitized guinea pigs. (*A*) Increase in lung resistance (R_L) in response to intravenous ACh in control animals (CONTR+VEH) and ovalbumin-sensitized and challenged animals (OA/OA+VEH), and the effect of wortmannin (1 mg/kg, intranasal) pretreatment (OA/OA+WTM). (*B*) As in (*A*), but with intravenous histamine as the spasmogen. Values represent means ± SEM, n = 5 or 6. The baseline R_L was 255.4 ± 8.6 cm H_2O/L per s and no significant difference was seen among the various groups. *$p < 0.05$, compared with CONTR+VEH; #$p < 0.05$, ##$p < 0.01$, compared with OA/OA+VEH.

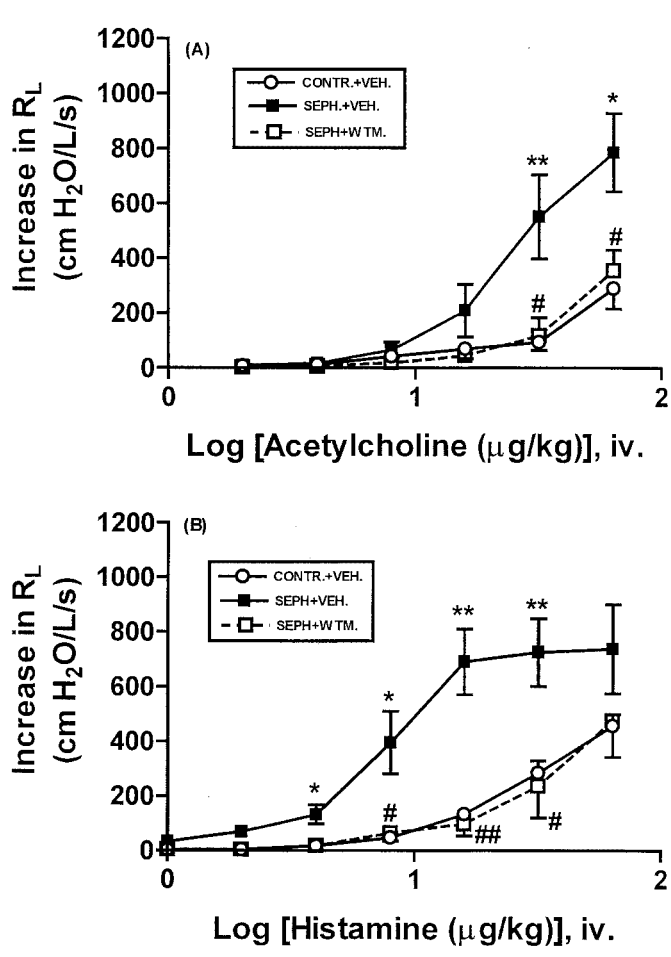

Figure 4. Effect of intranasally administered wortmannin (WTM) or drug vehicle (VEH) on the development of AHR, 24 h after injection of Sephadex beads (SEPH) in guinea pigs. (*A*) Increase in lung resistance (R_L) in response to intravenous ACh in control animals (CONTR+VEH) and Sephadex-treated animals (SEPH+VEH), and the effect of wortmannin pretreatment animals (SEPH+WTM). (*B*) As in (*A*), but with intravenous histamine as the spasmogen. Values represent means ± SEM, n = 5, in all groups. The overall baseline R_L was 255.4 ± 8.6 cm H_2O/L per s and no significant difference was seen among the various groups. *$p < 0.05$, **$p < 0.01$, compared with CONTR+VEH; #$p < 0.05$, ##$p < 0.01$, compared with SEPH+VEH.

AHR to histamine was almost completely abolished by the treatment. In four or five experiments, neither the vehicle (10% DMSO, 400 μl, intranasal) nor wortmannin (1 mg/kg, intranasal) had any significant effect on airway responses or the baseline R_L to ACh and histamine in control animals (data not shown).

Sephadex-injected guinea pigs also developed, within 24 h, a pronounced AHR to both ACh and histamine, reflected as a leftward shift in the dose–response curve of the two spasmogens (Figure 4A and 4B). For example, the increase in R_L to ACh at 32 μg/kg was 93.2 ± 30.0 cm H_2O/L per s (n = 5) in control animals compared with 551.7 ± 158.8 cm H_2O/L per s (n = 5) in Sephadex-injected animals (both pretreated with the drug vehicle), $p < 0.01$. For histamine (16 μg/kg), the increase was from 133.6 ± 41.5 cm H_2O/L per s (n = 5) in the controls to 690.0 ± 119.6 cm H_2O/L per s (n = 5) in Sephadex-injected animals, $p < 0.01$. Pretreatment with wortmannin (1 mg/kg, intranasal) completely abolished the AHR to both spasmogens.

Correlation Analysis of AHR with BALF Eosinophilia and EPO Activity

As seen in Figure 5A and 5B, among all animals in which bronchial inflammation was induced with allergen, no significant correlation was found between BALF eosinophil count and AHR to both ACh and histamine as judged by the increase in R_L to a 32-μg/kg concentration of both spasmogens. In contrast, BALF EPO activity positively correlated with AHR to both spasmogens (ACh: $r = 0.4847$, $p = 0.0415$; histamine: $r = 0.4936$, $p < 0.0374$). For the Sephadex model, a similar pattern of correlation was seen (Figure 5C and 5D).

DISCUSSION

Many inflammatory mediators attract and activate eosinophils via transduction pathways involving the wortmannin-sensitive PI 3-kinase enzyme, and wortmannin has been shown to be a potent inhibitor of a number of eosinophil responses *in vitro* (8–13). Initially, we sought to confirm the direct effect of wortmannin on both human and guinea pig eosinophils *in vitro*. The result obtained not only confirmed the high potency of this drug in inhibiting O_2^- release from eosinophils of both species, but also showed, for the first time, that the drug was equally a potent inhibitor of human eosinophil degranulation. In view of these observations, and given that eosinophils play important roles in the pathophysiology of asthma, we set out to determine whether the drug would be effective in inhibiting bronchial eosinophilic inflammation and AHR—parameters that characterize clinical asthma. Because of the ubiquitous nature of PI 3-kinase in the signal transduction pathway of many cell types (7, 21–23), we chose to administer the drug by intranasal instillation in order to localize the effect to the lungs. Drugs administered by this route in guinea pigs have been shown to reach the bronchial tree easily (24). The results obtained have shown, for the first time, that intranasally administered wortmannin, in as low a dose as 1 mg/kg, could inhibit both allergen- and Sephadex-induced AHR *in vivo*. In detail, the results show some interesting points about this drug. First, in allergen-induced lung inflammation, the drug completely failed to prevent the increase in eosinophil influx into the lung, but significantly attenuated the increase in BALF EPO content (index of bronchial eosinophil degranulation) and AHR to histamine, but surprisingly not to ACh. This might suggest that it is the degranulation of eosinophils in the lungs, rather than their mere accumulation, which is important for the development of allergen-induced AHR to histamine. Others (25–28) have reported a similar dissociation between AHR and pulmonary eosinophilia in allergen-sensitized and challenged guinea pigs. In patients with asthma, however, BALF eosinophil count has often been positively correlated with the magnitude of AHR (29, 30).

Second, in contrast to the situation in allergic model, wortmannin almost completely abolished Sephadex-induced BALF eosinophil accumulation, in addition to significantly reducing BALF EPO levels and abolishing AHR. In both models, however, it is the EPO levels, not BALF eosinophil numbers, that

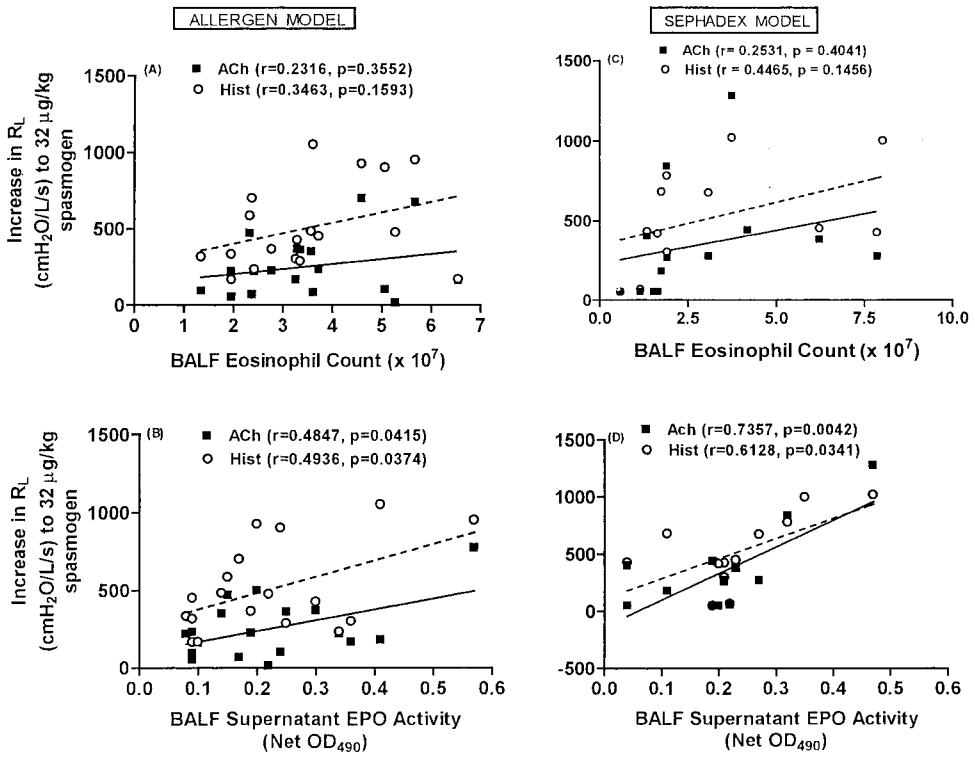

Figure 5. Correlation of AHR with the eosinophil count and eosinophil peroxidase activity (EPO) in BALF for all animals in which inflammation was induced with allergen (*A* and *B*) or Sephadex (*C* and *D*). AHR was based on increase in lung resistance (R_L) produced by ACh (32 μg/kg, intravenous) or histamine (32 μg/kg). r = Pearson's correlation coefficient.

correlated with the magnitude of AHR. This further supports the view that eosinophil degranulation plays an important role in the development of AHR in this species, as reported by others (31, 32). It has been hypothesized that the activation of eosinophils, and their subsequent release of cytotoxic cationic proteins, contribute substantially to the bronchial epithelial damage and the consequent AHR (5, 6, 33). In view of this hypothesis, one observation that is difficult to explain is why wortmannin was effective in inhibiting allergen-induced AHR to histamine, but not to ACh, in the same animals in which the drug inhibited EPO release. This may be related to the magnitude of the reflex component of their bronchoconstriction, which is greater for histamine than for ACh. A similar spasmogen-selective AHR has been reported in sensitized guinea pigs challenged with allergen (34). It is possible that in our study a higher dose of wortmannin or a longer duration of pretreatment was necessary to affect AHR to ACh. This would, perhaps, be consistent with the fact that the drug showed a trend toward reduction of AHR to ACh. There was also a positive correlation between BALF EPO levels and AHR to ACh in this model, even though the inhibition of EPO release by wortmannin was not accompanied by a significant inhibition of AHR to ACh. Furthermore, it should be realized that only at the highest dose of wortmannin tested (1 mg/kg) was a statistically significant inhibition of BALF EPO levels obtained, suggesting that this dose was the threshold of effectiveness. Unfortunately, the limitation posed by drug solubility, and the need to avoid vehicle effect that would likely occur with greater than 10% DMSO, did not allow us to test higher doses by this route of administration.

Third, the drug clearly distinguished between allergen- and Sephadex-induced eosinophil accumulation by significantly inhibiting this response when induced by the latter, but not the former. This would suggest an important difference in the mediators or mechanisms mediating eosinophil chemotaxis in the two models. Unlike in allergen-induced eosinophil accumulation, for which several eosinophil chemotactic mediators such PAF, IL-5, and eotaxin are known to be involved (35–37), the mediators involved in Sephadex-induced lung eosinophil accumulation in guinea pigs are less well defined although IL-5 (38) and complement activation products (39) have been proposed. Differential mediator involvement may also contribute to the differences in the ability of wortmannin to inhibit AHR to ACh in the two models.

Because the inhibition of AHR by wortmannin in both models was invariably accompanied by significant inhibition of EPO release, and given that in both models the magnitude of AHR positively correlated with BALF EPO levels, but not eosinophil count, it could be concluded that wortmannin inhibited AHR by virtue of its ability to inhibit eosinophil degranulation. However, because the drug could abolish AHR even when it reduced the elevated BALF EPO levels by only about 50%, it is possible that other eosinophil-independent mechanisms may contribute to its ability to prevent AHR. Wortmannin-sensitive PI 3-kinase enzyme appears to play signal transduction roles in many cell types other than eosinophils (7, 21–23). Although the intranasal route of administration adopted in this study may have ensured that systemic effects were minimized, many other cell types within the lungs may have been targets as well. For example, the drug may have inhibited the activity of resident T cells, macrophages, or epithelial cells, preventing their release of inflammatory cytokines and neurokinins that may contribute to the development of AHR (40–42).

In view of the high potency with which wortmannin inhibited human eosinophil degranulation *in vitro*, is it likely that the drug will exhibit such activity *in vivo* in humans. If that is the case, it would be reasonable to expect that it would prevent the development of allergic as well as nonallergic AHR in humans, as has been seen in guinea pigs in this study. This possibility would be consistent with the close relationship that is known to exist between bronchial eosinophil degranulation and AHR in individuals with asthma (3–5, 30).

In summary, wortmannin has a direct inhibitory effect on guinea pig and human eosinophils *in vitro*. When given by intranasal instillation to guinea pigs, it significantly inhibited allergen-induced AHR in a spasmogen-selective manner, but without affecting BALF eosinophilia. In the Sephadex model, it inhibited all parameters assessed. The ability to prevent AHR appears to be due, at least in part, to the inhibition of degranulation of bronchial eosinophils rather than the prevention of their accumulation in the lungs. These results are relevant to the possible clinical application of this class of drug in the treatment of airway diseases characterized by eosinophilic inflammation and AHR.

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