

The effect of repirinast on airway responsiveness to methacholine and allergen

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Repirinast, a novel ingested antiallergic asthma medication from Japan, was compared versus placebo on airway responsiveness to methacholine and was compared versus placebo and cromolyn on airway responses to allergen. In 14 patients with mild, stable, atopic asthma, we performed a double-blind, double-dummy, random-order trial with ingested repirinast 300 mg twice daily for 7 days, inhaled cromolyn 40 mg spincaps single dose, and double placebo on allergen-induced early (EAR) and late (LAR) asthmatic responses and increased airway responsiveness. In the 14 subjects, no difference occurred in methacholine PC₂₀ after 6 days of repirinast or 6 days of placebo. In the 13 subjects who completed the allergen study, single-dose cromolyn significantly reduced the EAR by 63% and the LAR by 65% versus placebo ($p < 0.02$); repirinast was not significantly different from placebo, both the EAR and LAR being reduced by less than 10%. Allergen-induced increase in methacholine responsiveness was borderline ($p = 0.052$), and no significant drug effects occurred. In these models, a 1-week treatment period with repirinast, like other oral antiallergic asthma medications (e.g., ketotifen, fumarate), provides no protection against airway responses to methacholine or allergen. (J ALLERGY CLIN IMMUNOL 1992;90:782-8.)

Key words: Repirinast, allergen responsiveness, methacholine responsiveness

Repirinast is an ingested "antiallergic" asthma drug currently used in Japan. This chemical, isopentyl 5,6-dihydro-7,8-dimethyl-4,5-dioxo-4H-pyrano [3,2-c] quinoline-2-carboxylate, is a "prodrug," and is deesterified to the active component.¹ It has antiallergic properties as demonstrated by inhibition of antigen-induced mediator release (histamine, SRS-A) from mast cells and both animal and human lung slices *in vitro*.²⁻⁶ *In vivo* animal studies show inhibition of antigen-induced respiratory depression and inhibition of passive cutaneous anaphylaxis.^{2, 7, 8} These effects are qualitatively similar to cromolyn but in some studies exceed the effects of cromolyn.²⁻⁸ In humans with asthma, it has been reported to be efficacious as evaluated by somewhat subjective global symptom scores.^{1, 9} Repirinast is reasonably well tolerated causing occasional gastrointestinal upset and minor hepatic dysfunction or skin rashes.¹ This agent has recently

Abbreviations used

FEV₁: Forced expiratory volume in 1 second
LAR: Late asthmatic response
EAR: Early asthmatic response

been approved for investigation in Europe and North America. In this investigation we assessed the effect of a 6-day course of repirinast on airway responsiveness to methacholine in a double-blind, placebo-controlled trial in 14 patients with mild, stable, atopic asthma. In 13 of the same subjects we also assessed the effect of a 7-day course of repirinast on airway responsiveness to allergen and compared it with a single dose of cromolyn sodium using a double-blind, double-dummy approach.

METHODS

Subjects

Fourteen atopic asthmatic subjects were recruited from the Chest Clinic at the Royal University Hospital in Saskatoon and from advertisements. Subjects had atopic asthma, which was, at the time of study, mild and well controlled with either no medication or occasional inhaled beta agonist. Forced expiratory volume in 1 second (FEV₁) was greater than 80% predicted, and subjects all had, by history, at least one allergen to which they responded both clinically and on allergy prick skin tests. Anthropometric

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Supported by a grant from Miles Pharmaceuticals Canada Limited. Received for publication March 19, 1992.

Revised June 5, 1992.

Accepted for publication June 5, 1992.

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1/1/40444

TABLE I. Anthropometric data, airway function, and allergen data

Subject	Age (yr)	Sex	Height (cm)	FEV ₁		Methacholine PC ₂₀ (mg/ml)	Rx*	Allergen	
				L/sec	(%)			Name	Dilution
1	27	F	165.74	3.36	105	2.7	S	Horse	1:64
2	27	M	181.00	3.63	82	1.1	F	Grass	1:128
3	26	M	175.26	4.17	98	12.0	O	Grass	1:1024
4	18	M	179.07	5.80	125	2.1	S	Grass	1:256
5	44	M	170.18	2.60	74	0.49	F	Grass	1:2048
6	27	F	167.64	2.80	86	1.2	S	<i>D. farinae</i>	1:32
7	33	M	170.18	3.19	83	2.1	S	Cat	1:64
8	30	M	171.45	5.58	140	2.9	S	<i>D. farinae</i>	1:16
9	23	F	160.02	3.50	113	8.1	O	Mixed grass	1:256
10	19	F	156.85	2.68	87	3.1	S	Horse	1:128
11	27	F	158.75	3.19	108	3.6	S	Cat	1:16
12	25	M	170.18	3.45	84	2.5	O	Cat	1:64
13	24	M	177.80	4.78	109	64.0	O	Cat	1:8
14	26	M	180.34	4.20	95	0.89	S	Horse	1:256

*S, Salbutamol p.r.n., F, fenoterol p.r.n.

data on the 14 subjects are shown in Table I. This investigation was approved by the President's Ethics Committee of the University of Saskatchewan, and signed informed consent was obtained.

Methacholine inhalation test

Airway responsiveness to methacholine was measured with use of a minor modification of the previously reported method.¹⁰ A Bennet Twin Jet nebulizer (Puritan Bennett Corp., Carlsbad, Calif.) (in place of a Wright nebulizer) was used. The nebulizer was calibrated to deliver an output of 0.13 ml/min, with an airflow rate of 4.5 L/min. Three milliliters of room temperature solutions were added to the nebulizer. FEV₁ was initially measured in triplicate. Isotonic saline solution was then inhaled for 2 minutes of tidal breathing, and the FEV₁ was repeated at 30 and 90 seconds. Thereafter, doubling concentrations of methacholine from as low as 0.03 mg/ml to as high as 64 mg/ml were then inhaled for 2 minutes at 5-minute intervals, and the FEV₁ was repeated at 30 and 90 seconds and if necessary, 180 seconds, after each inhalation. Change in FEV₁ was measured from the lowest postsaline FEV₁ to the lowest post-methacholine FEV₁. The test was continued until the FEV₁ had fallen by 20% or until the top concentration had been administered. The methacholine PC₂₀ was then calculated by a linear interpolation formula of the last two data points.¹⁰

Allergen inhalation tests

Allergen inhalation tests were done as previously reported.¹¹⁻¹³ The first day was a control day. Aerosols were generated by a Wright nebulizer calibrated at a flow rate of 6.5 L/min to give an output of 0.13 ml/min. The nebulizer was connected to a Hans-Rudolph (Hans Rudolph, Inc., Kansas City, Mo.) valve, and aerosols were inhaled via a mouthpiece. The uninhaled nebulizate, and the exhalate

were trapped with two ventilator filters (BB-50T, Pall Biomedical Inc., Fajardo, Puerto Rico) on the expiratory side of the valve. After a reasonable rest period in the laboratory (several minutes to three quarters of an hour depending on the subject), FEV₁ was measured in triplicate. The allergen diluent, isotonic buffered saline with 0.5% phenol, was then inhaled for 2 minutes of tidal breathing on three occasions at 10-minute intervals. The FEV₁ was repeated in duplicate 10 minutes after each inhalation and after the third inhalation, every 10 minutes for the first hour, every 30 minutes for the second hour, and hourly up to 7 hours. On the control day, duplicate prick skin tests with the allergen inhalation were performed from 1:8 dilution to 1:1024 or greater dilution. The skin test end point was defined as the smallest concentration producing a mean wheal diameter (duplicate tests measured in two perpendicular directions) of 2 mm. Methacholine PC₂₀ was measured at 7 hours. The early asthmatic response (EAR) allergen PC₂₀ was predicted from the methacholine PC₂₀ and the skin test end point.¹³ Appropriate concentrations of allergen were frozen in multiple single-use 3 ml aliquots in Wright nebulizer-compatible vials on the basis of commencing allergen concentration (for safety sake and to allow a minimum of three concentration inhalations if possible), four or five concentrations below the prediction, or at the end point, whichever was highest.

On the second day, allergen inhalations were started either four or five concentrations below the prediction or at the skin test end point concentration. Doubling concentrations of allergen were inhaled for 2 minutes at 10-minute intervals, with the same inhalation system as for the control. FEV₁ was initially measured in triplicate and 10 minutes after each allergen concentration. Allergen inhalations were stopped when the FEV₁ had fallen 12% or greater; the highest preinhalation and highest postinhalation FEV₁s were

TABLE II. Effect of placebo and repirinast on methacholine PC₂₀

	Methacholine PC ₂₀ (mg/ml) Geometric mean		t	p
	Pre	Post		
Placebo	4.0	4.4	0.95	>0.20
Repirinast	3.8	3.3	1.12	>0.20

used to determine the percent change. FEV₁ was then measured for 7 hours as on the control day. Methacholine PC₂₀ was repeated 7 and 30 hours after allergen challenge.

Study design

Subjects attended the laboratory on a minimum of 16 occasions during a time when they were not exposed to allergen or respiratory tract infection for in excess of 4 weeks, when their asthma was stable and required no treatment other than occasional beta agonist, and when their FEV₁ was stable ($\pm 10\%$). The 16 days included 5 whole days in the laboratory 4 of which were allergen test days. Day 1 and day 2 were the screening control day and allergen test day as outlined herein.

On day 3 and day 4, allergen inhalation tests were repeated in exactly the same fashion with use of the last three concentrations of allergen that were administered on day 2. Days 3 and 4 were preceded by active treatments, repirinast for 7 days and sodium cromoglycate single dose administered in a double-blind, double-dummy, random-order fashion. During one period, subjects received repirinast 300 mg (2×150 mg tabs) twice daily for 7 days (14 doses) up to and including 1 hour before the allergen inhalation test, and a single dose of two placebo spincaps 10 minutes before allergen challenge. During the other period, they received placebo twice daily for 7 days and a single administration of 2×20 mg cromolyn sodium spincaps 10 minutes before allergen inhalation. Day 5 was an allergen test day repeated after single-blind administration of two placebo tablets twice daily for 7 days and two placebo spincaps 10 minutes before. There was at least a 1-week interval between completion of an allergen challenge and commencement of ingested medications, indicating a minimum 2-week interval between allergen challenges and a minimum total time span of 6 weeks between days 2 and 5.

A summary of the study design is as follows.

Day 1: Saline control day.

Day 2: Allergen challenge.

Days 3 and 4: Allergen challenges after active treatments; seven doses repirinast and one dose cromolyn double-blind, double-dummy, random order.

Day 5: Allergen challenge after double placebos.

Methacholine PC₂₀ was measured on four occasions in relationship to each of the day 3, 4, and 5 allergen challenges: before commencing the ingested medications (within

$\pm$ one doubling concentration of baseline values), after 6 days (12 doses) of medication (the day before allergen challenge), 7 hours after, and 30 hours after allergen challenge.

Thirty minutes after the last dose of ingested medications, one half hour before the allergen challenge, on days 3, 4, and 5, blood was drawn. On all 3 days blood was sent for measurement of repirinast blood levels, hematologic, renal, and hepatic function. In women, a pregnancy test was done before the study and with the blood work on day 5; all pregnancy tests were negative.

Analysis

The EAR was defined as the maximum percent reduction in FEV₁ occurring within the first hour after allergen challenge. The late asthmatic response (LAR) was defined as the maximum percent reduction in FEV₁ between 3 and 7 hours after allergen inhalation. Allergen-induced increase in airway responsiveness was defined as the $\Delta \log PC_{20}$ from preallergen to postallergen; all methacholine PC₂₀ values were analyzed by log transformation.

The effect of repirinast and placebo on airway responsiveness to methacholine was analyzed with use of the data from the week before days 3 and 4. Pretreatment and post-treatment values for (log) methacholine PC₂₀ were compared by means of the paired *t* test.¹⁴ The change in log PC₂₀ after the two treatments was also compared by paired *t* test.

Analysis of the treatment effects of the three treatments (repirinast, sodium cromoglycate, and placebo) on EAR, LAR, and $\Delta \log PC_{20}$ from days 3, 4, and 5 were done with use of analysis of variance (ANOVA)¹⁵; individual significances were assessed by means of Scheffe's test.¹⁶

RESULTS

Thirteen subjects completed the study. One subject was dropped from the study before completion of all allergen challenges because of unexplained wide variability in the early asthmatic response to allergen on days 2, 3, and 5; the subject did not perform a day 4 challenge. Since days 3 and 5 were repirinast and placebo, respectively, in this subject, his data were included in analysis of the effect of repirinast on methacholine PC₂₀. Three subjects had mild-to-moderate gastrointestinal upset while taking repirinast compared with one taking placebo (before the sodium cromoglycate day). No changes occurred in hematologic, renal, or hepatic function during repirinast treatment. Repirinast blood levels varied between 43 and 1115 ng/ml (median, 181) on the repirinast day and were undetectable in all subjects on the cromolyn and placebo days.

The effect of a 6-day treatment with repirinast and placebo on methacholine PC₂₀ is summarized in Table II. The geometric mean methacholine PC₂₀ varied between 3.3 mg/ml and 4.4 mg/ml. There were no significant differences after either treatment, and the change in log PC₂₀ after repirinast (-0.07 ± 0.24)

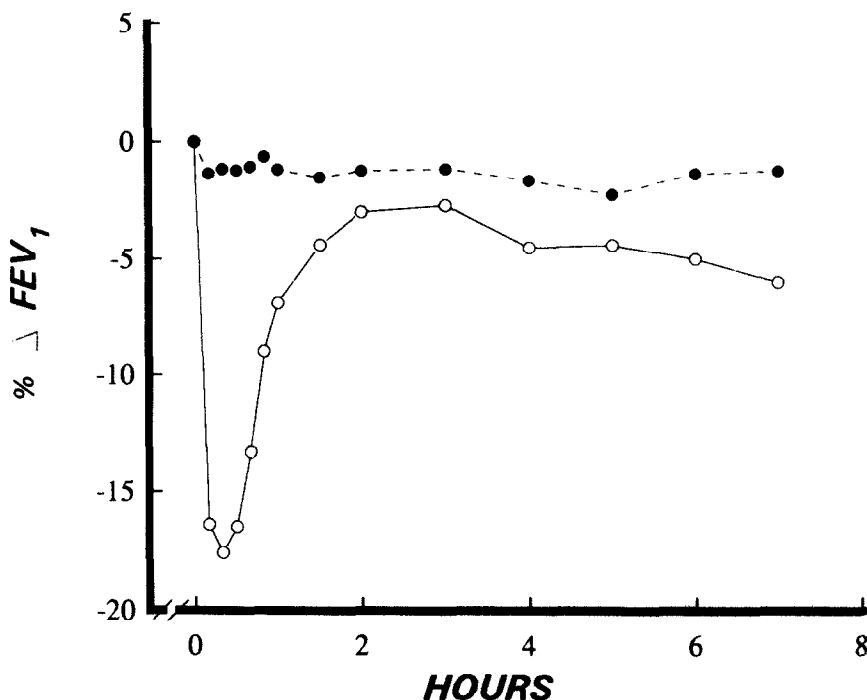


FIG. 1. Control and allergen inhalation day. Percent Δ FEV₁ on vertical axis and time in hours after completion of allergen challenge on horizontal axis. Solid circles represent day 1 diluent inhalation, open circles represent day 2 allergen inhalation.

was not significantly different from that after placebo ($+0.05 \pm 0.19$) paired $t = 1.23$, $p > 0.02$.

No significant effect of treatment occurred on baseline FEV₁ ($p > 0.05$). The mean values for FEV₁ (% Δ) after allergen challenges are shown in the 13 subjects in Figs. 1 and 2. No significant change occurred in FEV₁ after the diluent control inhalation (Fig. 1). All subjects had an EAR on day 2 (Table III) ranging from 12% to 28% ($20.6\% \pm 5.2\%$). Five of the 13 subjects had LARs greater than 10%; the mean LAR for the entire group was $9.2\% \pm 8.5\%$. The reproducibility of the allergen challenge was compared on days 2 and 5 (allergen alone vs allergen and placebo). The early asthmatic response (varied less than $\pm 50\%$ in 9 of the 13 subjects and showed greater than 50% variability, all in the direction of smaller response on day 5 in the remaining four (Table III). The LAR varied less than $\pm 50\%$ in four of five; one isolated early responder on day 2 had an LAR on day 5 (Table III). The interclass correlation coefficients calculated from days 2 and 5 were 0.101 and 0.62 for EAR and LAR, respectively.¹⁷ Individual repirinast levels are also shown in Table III; no apparent relation was observed between level and apparent inhibition of EAR or LAR.

The effect of drug treatments on allergen-induced

responses is shown in Fig. 2. The mean allergen-induced EARs and LARs are virtually identical for placebo and repirinast, and both are substantially lower (by 63% and 65%, respectively, than placebo) on the cromolyn day. Analysis of variance revealed a significant treatment effect on both the EAR ($p = 0.0019$) and the LAR ($p < 0.015$). Both EARs and LARs after cromolyn were significantly different (smaller) than after either of the other two treatments; repirinast did not differ from placebo. No apparent dose-response effect was seen when repirinast blood levels were compared with possible protective effect(s).

Allergen-induced fall in methacholine PC₂₀ (Fig. 3) was only marginally significant at 7 hours on the placebo day ($t = 2.13$, $p = 0.052$). There was no significant treatment effect on $\Delta \log PC_{20}$.

DISCUSSION

In 14 patients with mild, stable, atopic asthma, repirinast 300 mg twice daily for 6 days failed to alter airway responsiveness to methacholine. In 13 of these 14 asthmatic patients, repirinast 300 mg twice daily for 7 days had no effect on the EAR or LAR to allergen, whereas a single 40 mg dose of sodium cromoglycate significantly inhibited both EARs and

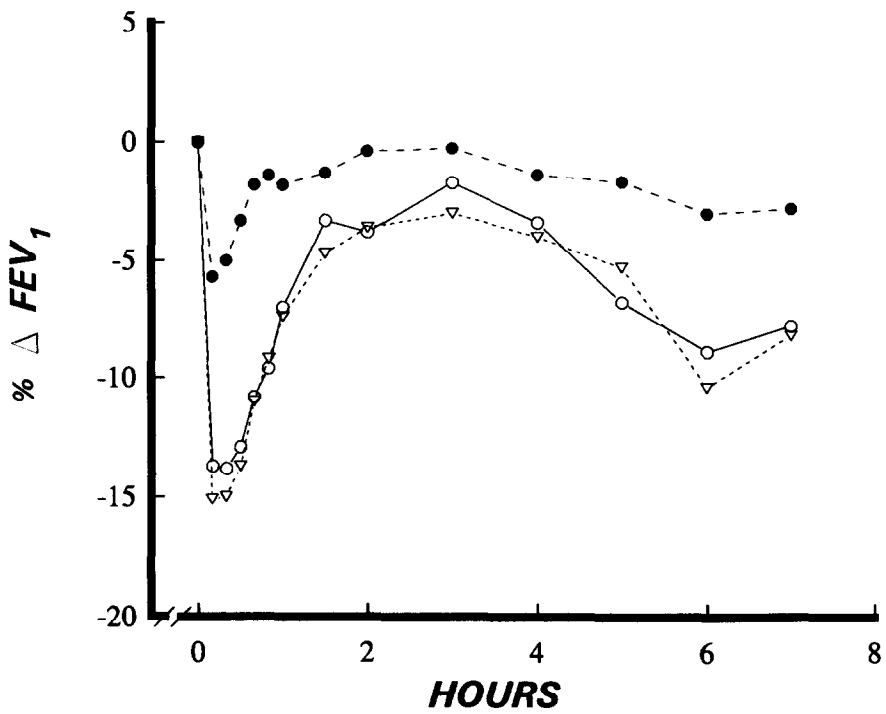


FIG. 2. Allergen challenges on days 3, 4, and 5. Percent change in FEV₁ on vertical axis, time in hours after completion of allergen challenge on horizontal axis. Allergen response after sodium cromoglycate shown in solid circle, after repirinast in open circles, and after placebo in open triangles.

TABLE III. Early and late responses and repirinast blood levels

Subject no.	EAR (% Δ FEV ₁)				LAR (% Δ FEV ₁)				Repirinast level (ng/ml)
	Day 2	Days 3 and 4		Day 5	Day 2	Days 3 and 4		Day 5	
		Repirinast	Cromolyn	Placebo		Repirinast	Cromolyn	Placebo	
1	21.9	6.0	6.3	12.1	6.3	3.0	0	3.0	1035
2	20.6	9.6	5.6	4.3	11.1	6.8	2.8	5.8	377
4	24.8	29.0	3.8	35.8	7.3	32.7	8.7	34.9	46
5	26.1	4.3	22.2	17.0	26.1	8.7	0	31.9	66
6	21.7	25.5	0	33.3	4.3	17.0	7.0	8.3	292
7	22.0	14.0	5.7	25.4	22.0	28.1	0	33.3	181
8	12.0	23.5	13.0	16.0	17.0	20.6	16.0	9.0	1115
9	22.7	22.1	10.3	30.3	2.0	4.4	2.9	7.6	43
10	24.5	12.5	0	6.0	0	0	0	0	52
11	16.1	29.3	8.9	26.2	1.8	13.8	8.9	9.8	173
12	15.2	13.4	9.1	4.5	7.6	4.5	7.6	9.0	430
13	12.0	4.4	2.3	15.1	0	2.2	1.2	5.8	110
14	28.0	14.8	0	8.6	14.6	0	0	2.4	181
mean	20.6	16.0	6.7	18.0	9.2	10.9	4.2	12.4	
SD	5.2	9.0	6.2	11.1	8.5	10.8	5.0	12.3	

LARs. In these subjects, not specifically selected to demonstrate allergen-induced increase in airway responsiveness, only borderline increase occurred in airway responsiveness to methacholine after allergen,

and no significant treatment effect of either treatments could be found.

Limited data are available on the effectiveness of this ingested antiallergic drug. Studies in Japan, gen-

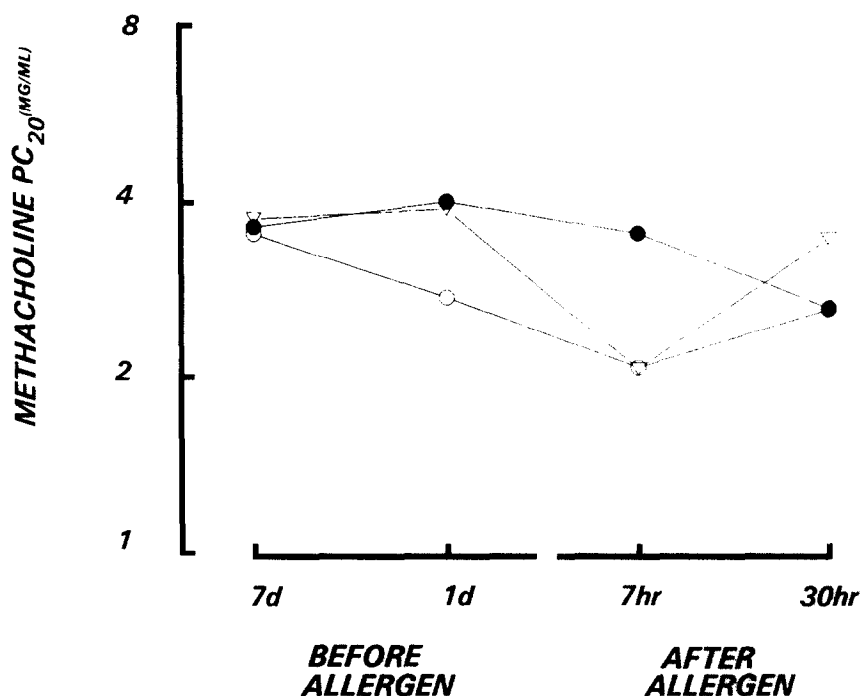


FIG. 3. Geometric mean methacholine PC_{20} (mg/ml, vertical axis) 7 days before, 1 day before, 7 hours after, and 30 hours after allergen inhalation. Solid circles sodium cromoglycate day, open circles repirinast day, triangles placebo (day 5).

erally open, appear to suggest modest efficacy as assessed by subjective criteria.¹⁻⁹ In an unblinded, non-random-order trial, a single 270 mg ingested dose of repirinast was found to be effective in inhibiting allergen-induced EARs in slightly less than 50% of subjects (14 of 29) and to inhibit cutaneous wheal and flare responses to allergen in 20% (6 of 30).¹⁸ The blood levels in our study (40 to 1000 ng/ml = 0.4×10^{-7} to 10^{-6} gm/ml) were similar to levels (10^{-6} to 10^{-7} gm/ml), which have proved effective in vitro to inhibit allergen-induced mediator release.²

Allergen-induced increase in airway responsiveness may be the best indirect measure of allergen-induced airway inflammation.¹⁹ This may be particularly useful for testing the "antiinflammatory" effect of asthma treatment. It is probably a better indirect measure of airway inflammation than is the LAR, since the latter is significantly reversible with bronchodilator and may be masked rather than truly inhibited.¹⁹ In previous studies we have shown that allergen-induced increase in airway responsiveness can be inhibited by single dose pretreatment with sodium cromoglycate,^{11,12} and beclomethasone¹¹ but not by salbutamol,¹¹ theophylline,¹² ketotifen,²¹ or clemastine.²¹ Unfortunately, our selection of "all comers" for allergen challenges in this study resulted in a large range of LARs (the majority of the subjects were not late responders), and of $\Delta \log PC_{20}$ values, and this, accompanied by the within-subject variability, effectively nullified our op-

portunity to look at allergen-induced increase in airway responsiveness. Although masking of the late response by a bronchodilator (e.g., theophylline) might be associated with no improvement in allergen-induced airway hyperresponsiveness,¹² it might be reasonable to expect that failure of inhibition of the late response (e.g., salbutamol, ketotifen, clemastine) would be associated with no inhibition of allergen-induced increases in airway responsiveness. However, in one study in seven subjects, indomethacin was shown to at least partially inhibit $\Delta \log PC_{20}$ without influencing either the EAR or the LAR.²²

It is interesting to note that short course ketotifen, another widely used ingested "antiallergic" asthma therapy, when compared with clemastine, cromolyn, and placebo, was equally ineffective in this allergen challenge model.²²

This investigation has given us further insights into the use of allergen inhalation tests in evaluation of asthma pharmacology. The first observation is that the longer the time interval between allergen challenges, the less reproducible they are. On occasion, it appeared that this nonreproducibility might be associated with gradual slight improvement in methacholine airway responsiveness leading to reduction in the EAR. In most subjects, however, the nonreproducibility of the early response was unexplained. Subject no. 3 had such wide unexplainable variability in the early response that we felt we had to exclude him from con-

tinuing in the trial. This subject initially had a 19% FEV₁ fall with grass pollen 1:1024. On the repirinast day (day 3), he had a 41% FEV₁ fall after grass pollen 1:4096. On the placebo day (day 5), which was done out of order specifically to check reproducibility, he had only a 5% FEV₁ fall at an allergen concentration of 1:1024. Airway responsiveness, FEV₁, and symptoms were stable at the time of the three allergen challenges. This represents a variability of almost 32-fold in the early asthmatic PC₂₀; this is the largest unexplained variation in early response that we have ever witnessed. These observations provide the rationale for attempting to keep the time span of a series of allergen challenges to the minimum to appropriately complete the investigation, and also provide the rationale for the use of two unmedicated allergen challenges to check individual reproducibility. When we are investigating two or more agents compared with placebo, it has been our practice to do the screening allergen challenge day on day 2 and the single-blinded placebo day as the last study day.^{11, 12, 21} Where only one agent is being compared with placebo, the active drug and placebo have been administered double-blind, random-order on days 3 and 4.²²

The second observation is one of patient selection. With careful patient selection, we have been able to study allergen-induced increase in airway responsiveness and late responses in 6 to 12 individuals.^{11, 12, 21, 22} Inclusion of the more heterogeneous population, as noted above, negated the opportunity to examine this important aspect.

In conclusion, we have demonstrated that a 6- to 7-day course of repirinast failed to significantly influence airway responsiveness to methacholine or the EARs or LARs to allergen in patients with mild, stable, atopic asthma. Allergen-induced increase in airway responsiveness could not be assessed in this trial.

The authors thank Joe Mink and Jacquie Bramley for their assistance in the preparation of this manuscript.

REFERENCES

1. Anon (Mitsubishi Ltd., Japan). Repirinast. *Drugs of the future* 1987;12:37-43.
2. Takahashi K, Shono T. The effect of MY-5116 on the PCA reaction and on histamine release from peritoneal cells induced by antigen-antibody reactions. *Folia Pharmacol Jpn* 1986;87:29-39.
3. Takahashi K, Shono T, Arai Y. Effects of MY-1250, a main active metabolite of MY-5116, a new anti-allergic drug, on histamine release from rat peritoneal exudate cells and the degranulation of mesenteric mast cells. *Jpn J Allergol* 1986;35:1028-36.
4. Kohno S, Ohata K. Antiallergic effect of MY-5116 and its major metabolite, MY-1250. *Jpn J Allergol* 1986;35:1105-12.
5. Takahashi K, Yamada N, Endoh K, Yasumura K. Effects of MY-1250, a main active metabolite of MY-5116, a new anti-allergic agent, on the release of mediators from guinea pig lung fragments. *Jpn J Allergol* 1986;35:982-7.
6. Takahashi K, Shono T. Effects of MY-1250, a main active metabolite of MY-5116, a new anti-allergic drug, on histamine release from monkey lung fragments. *Jpn J Allergol* 1986;35:975-81.
7. Takahashi K. Effects of MY-5116 on experimental asthma in rats and guinea pigs. *Jpn J Allergol* 1986;35:1037-46.
8. Takashi K, Endoh K, Yamada N, Kadowaki S, Arai Y, Sugawara K. Effects of MY-5116 on experimental animal models of type I to type IV allergic reactions and on the formation of IgE antibody. *Folia Pharmacol Jpn* 1986;88:245-54.
9. Makino S, Takishima T, Takahashi S, Kabe J, Shida T. Clinical study of effect on MY-5116 on adult bronchial asthma early phase II study. *Medical consultative and new remedies (Shinryo to Shinyaku)* 1986;23(suppl):21-33.
10. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
11. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses and increased bronchial responsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
12. Cockcroft DW, Murdock KY, Gore BP, O'Byrne PM, Manning P. Theophylline does not inhibit allergen-induced increase in airway responsiveness to methacholine. *J ALLERGY CLIN IMMUNOL* 1989;83:913-20.
13. Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;135:264-7.
14. Steel RDG, Torrie JH. *Principles and procedures of statistics: a biometrical approach*, 2nd ed. New York: McGraw-Hill, 1980:102-4.
15. Steel RDG, Torrie JH. *Principles and procedures of statistics: a biometrical approach*, 2nd ed. New York: McGraw-Hill, 1980:137-71.
16. Steel RDG, Torrie JH. *Principles and procedures of statistics: a biometrical approach*, 2nd ed. New York: McGraw Hill, 1980:183-4.
17. Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation. *Chest* 1983;84:505-6.
18. Shinoda T, Takishima T, Mue S, et al. Evaluation of inhibitory effect of MY-5116 on house dust inhalation-induced reactions and intracutaneous reactions in allergic-patients. *Medical Consult New Rem (Shinryo to Shinyaku)* 1986;23(suppl):15-20.
19. Cockcroft DW. Allergen-induced airway hyperresponsiveness: a model for examination of "anti-inflammatory effects of asthma treatment." In: Morley J, ed. *Preventive therapy in asthma*. London: Academic Press, 1991:141-52.
20. Dorsch W, Baur X, Emslander HP, Fruhmant G. Zu Pathogenese und Therapie der allergeninduzierten verzögerten Bronchialobstruktion. *Prax Pneumol* 1980;34:461-8.
21. Cockcroft DW, Keshmiri M, Murdock KY, Gore BP. Allergen-induced increase in airway responsiveness is not inhibited by acute treatment with ketotifen or clemastine. *Ann Allergy* 1992;68:245-50.
22. Kirby JG, Hargreave FE, Cockcroft DW, O'Byrne PM. Indomethacin inhibits allergen-induced airway hyperresponsiveness but not allergen-induced asthmatic responses. *J Appl Physiol* 1989;66:578-83.