Different Effects of Inhaled Aspirinlike Drugs on Allergen-Induced Early and Late Asthmatic Responses

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Little is known about the anti-asthmatic effects of powerful anti-inflammatory agents such as aspirin-like drugs. We compared the effects of two aspirin-like drugs with different pharmacologic activities, sodium salicylate (SSA) and indomethacin, with the effect of lysine acetylsalicylate (LASA), inhaled 30 min before challenge, on the early and the late asthmatic response induced by a single dose of allergen causing a 25% decrease in FEV_1 in a preliminary challenge. Inhaled SSA partially prevented both the early and late response, providing a protection with respect to placebo of 22 ± 6% in the early phase and 23 ± 9% in the late phase of the response. These values were lower (but not significantly) than those of LASA (41 ± 9% and 39 ± 11%, respectively). In a second group of patients, indomethacin failed to affect the early response, while LASA provided a protection of 31 ± 7%. However, these two drugs were equally effective in reducing the late response (44 ± 18% and 39 ± 17% protection for LASA and indomethacin, respectively). In subjects with an early response, despite being ineffective in preventing allergen-induced bronchoconstriction, indomethacin blocked the allergen-induced increase in bronchial hyperresponsiveness measured 2 h after challenge. We conclude that inhaled salicylates, but not indomethacin, exert a protective activity against the early allergic response. This difference is not explained by the different pattern of cyclooxygenase inhibitory activity of these drugs. Sestini P, Refini RM, Pieroni MG, Vaghi A, Robuschi M, Bianco S. Different effects of inhaled aspirinlike drugs on allergen-induced early and late asthmatic responses.

AM J RESPIR CRIT CARE MED 1999;159:1228–1233.

Anti-inflammatory agents, especially corticosteroids, currently have a major role in the treatment of asthma (1, 2). However, despite their well-known anti-inflammatory activity (3), the effect of aspirin-like drugs on asthma has received little attention, mostly because of the negative or conflicting results obtained in experimentally induced bronchoconstriction. We and others have recently observed that when given by inhalation, a soluble form of aspirin, lysine acetylsalicylate (LASA), significantly inhibits the bronchial obstructive response induced by a variety of stimuli, including ultrasonically nebulized distilled water (UNW) (4), adenosine (5), neurokinin A (6), metabisulphite (7), histamine (8), and allergens (9).

The protective effect of LASA against experimentally induced bronchoconstriction appears to be shared by some, but not all, aspirin-like drugs. In the case of bronchoconstriction induced by UNW (4), metabisulphite (7), and exercise (10), inhalation of a single dose of indomethacin also significantly inhibited the response, whereas sodium salicylate had no significant effect against UNW- and metabisulphite-induced bronchoconstriction (4, 7). Since these drugs differ significantly in the mechanism of their anti-inflammatory action, this heterogeneity could throw some light on the mechanisms involved in the bronchoconstrictor response to these stimuli. The aim of the present study was to investigate whether the protective activity of aspirin-like drugs against allergen-induced bronchoconstriction also exhibits a heterogeneous pattern similar to the one observed with UNW and exercise.

METHODS

Patients

We studied patients with mild allergic asthma, in a stable condition, presenting an early or dual asthmatic reaction in a preliminary bronchial challenge with the relevant allergen. Most of the patients were untreated except for regular or occasional use of short-acting inhaled β_2-agonist. All treatments were withheld at least 14 h before each test. Patients' allergic responses to pollen were studied outside the pollen season. Patients with aspirin-induced asthma were excluded from the study, and for those who could not report having taken aspirin or other known cyclooxygenase inhibitors without side effects in the 12 mo preceding the study, a bronchial challenge with LASA (9) was performed to exclude aspirin sensitivity. All the patients performed a methacholine challenge as part of the preliminary clinical evaluation (11). The study was conducted according to the guidelines of the ethical committee of our institution, requiring informed consent from each patient.

(*Received in original form September 10, 1997 and in revised form July 8,1998*)

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TABLE 1

CLINICAL AND ANTHROPOMETRIC DATA OF SUBJECTS IN THE STUDY ON THE EFFECT OF INHALED SODIUM SALICYLATE

Patient No.	Age (*yr*)	Sex	Allergen	Bronchial Response	PD_{25}*	FEV_1†	Therapy
1	25	M	PA	Dual	0.3	84	β_2
2	28	M	PA	Dual	1.2	100	
3	19	M	GR	Dual	1.2	93	
4	18	F	DP	Dual	2.6	111	
5	23	M	DP	Dual	2.6	91	β_2
6	25	M	GR	Dual	2.4	95	
7	22	F	GR	Early	1.0	82	
8	18	F	GR	Early	0.3	99	β_2
9	19	M	GR	Early	0.2	91	β_2
10	20	M	GR	Early	2.4	100	
11	23	M	DP	Early	2.4	100	β_2 + CS
12	19	M	GR	Early	3.0	76	β_2
Mean ± SEM	22 ± 1					94 ± 3	

Definition of abbreviations: β_2 = β_2-agonist; CS = inhaled steroids; DP = Dermatophagoides; GR = grass pollen; PA = parietaria (nettles).

* Allergenic unit.

† Percentage of predicted at baseline.

TABLE 2

CLINICAL AND ANTHROPOMETRIC DATA OF SUBJECTS IN THE STUDY ON THE EFFECT OF INHALED INDOMETHACIN

Patient No.	Age (*yr*)	Sex	Allergen	Bronchial Response	PD_{25}*	FEV_1†	Therapy
1	22	M	PA	Dual	0.3	84	β_2
2	28	M	PA	Dual	1.2	100	
3	19	M	GR	Dual	1.2	93	
4	25	M	GR	Dual	0.5	103	
5	23	M	DP	Dual	2.5	91	β_2
6	25	M	GR	Dual	0.5	103	
7	24	M	GR	Dual	0.8	83	
8	26	M	PA	Dual	0.6	92	
9	41	F	DP	Early	0.9	81	β_2 + CS
10	25	M	GR	Early	1.6	88	
11	19	M	GR	Early	0.2	91	
12	23	M	DP	Early	2.4	100	β_2 + CS
13	18	M	GR	Early	3.0	80	
14	22	M	GR	Early	0.8	90	
15	24	F	GR	Early	3.2	93	
16	20	M	GR	Early	2.4	100	
Mean ± SEM	24 ± 1					92 ± 2	

For definition of abbreviations, *see* Table 1.

* Allergenic unit.

† Percentage of predicted at baseline.

Bronchial Challenges

Bronchial challenges with allergen (Frazioni Alfa; Dome/Hollister-Stier, Bayropharm Italiana, Milan, Italy) and methacholine (Mch) were carried out with a dosimeter (Mefar; Markos, Brescia, Italy), as previously described (11). In the allergen challenge, an early reaction was taken to be a decrease in FEV_1 of 20% or more with respect to baseline, between 15 and 30 min after challenge. The dose causing a 25% decrease in FEV_1 (PD_{25}), expressed in RAST activity units (AU), was computed by interpolation on the cumulated dose–response curve. The patients presenting an early response were then monitored for the rest of the day with a Mini-Wright peak flow meter, and a late response was taken to be a decrease in FEV_1 or peak flow rate (PEFR) of 15% or more between 4 and 8 h after challenge. Patients presenting a late response were subjected to PEFR monitoring during a control day and if a PEFR variation of more than 10% was observed, they were considered unstable and excluded from the study. All the experiments were conducted within 3 mo from the preliminary test.

Effect of Inhaled Sodium Salicylate and LASA on the Early and Late Asthmatic Reaction to Allergen Challenge

We studied 12 patients, as detailed in Table 1. Six of the patients had a dual response to a preliminary bronchial allergen challenge. Each patient performed three allergen challenges, using a single dose corresponding to the PD_{25} calculated in the preliminary test. Before each challenge the patients were treated, according to a randomized double-blind protocol, with either LASA 900 mg, corresponding to 500 mg of acetylsalicylate acid (ASA) (Flectadol; Maggioni Winthrop Italia, Milan, Italy), sodium salicylate (SSA) 500 mg, or placebo, administered by inhalation in 5 ml of saline with a jet nebulizer (Nebula; Markos, Monza, Italy). The challenge was performed 30 min after the end of nebulization. FEV_1 was measured before and after treatment and then 5, 10, 15, 20, 30, 45, and 60 min after challenge. In the six patients (no. 1–6) who presented a late response in the preliminary test, FEV_1 was also recorded every 60 min for 8 h.

Effect of Inhaled Indomethacin and LASA on the Early and Late Asthmatic Reaction to Allergen Challenge

Sixteen patients, whose clinical and anthropometric characteristics are reported in Table 2, participated in this part of the study. Six of these patients also participated in the previous study. Each patient performed three allergen challenges with the same modalities as detailed above, except that premedication consisted of either LASA 900 mg, indomethacin meglumine 77.2 mg (corresponding to 50 mg of indomethacin), or placebo.

Effect of Inhaled Indomethacin on the Allergen-Induced Bronchial Hyperreactivity

In an additional part of the study we investigated the effect of indomethacin on allergen-induced bronchial hyperreactivity in six patients presenting an isolated early reaction. The protocol of the study was similar to the above, except that only indomethacin and placebo were used and a methacholine challenge was performed 2 h after allergen administration on the two study days. The results were compared with those obtained in the preliminary methacholine challenge, performed within 10 d before any allergen challenge. The characteristics of the patients participating in this study are reported in Table 3. Two of the patients also participated in previous studies.

Statistical Analysis

The protective activity of LASA was expressed as a percentage of the effect of placebo according to the formula: (placebo − treatment)/placebo × 100, computed from either the maximum decrease in FEV_1 or the area under the curve (AUC) of the differences from baseline over time. Changes in Mch reactivity were expressed as doubling doses (DD) of Mch PD_{20}, computed as $\log_2(PD_{20}\,\text{control}) - \log_2(PD_{20}\,\text{treatment})$. Changes of baseline FEV_1 after treatment and on different days were evaluated by ANOVA. All other comparisons were evaluated with repeated measures ANOVA and the least significant difference (LSD) multiple range test for multiple comparison, when required.

TABLE 3

CLINICAL AND ANTHROPOMETRIC DATA OF SUBJECTS IN THE STUDY ON THE EFFECT OF INHALED INDOMETHACIN ON ALLERGEN-INDUCED BRONCHIAL HYPERREACTIVITY

Patient No.	Age	Gender	Allergen	PD_{25}	FEV_1
1	25	M	GR	1.6	88
2	23	M	DP	2.4	100
3	22	M	GR	0.8	95
4	19	M	GR	0.3	95
5	22	M	GR	0.8	93
6	57	M	GR	0.3	98
Mean	28 ± 6				83 ± 11

Definition of abbreviations: DP = Dermatophagoides; GR = grass pollen.

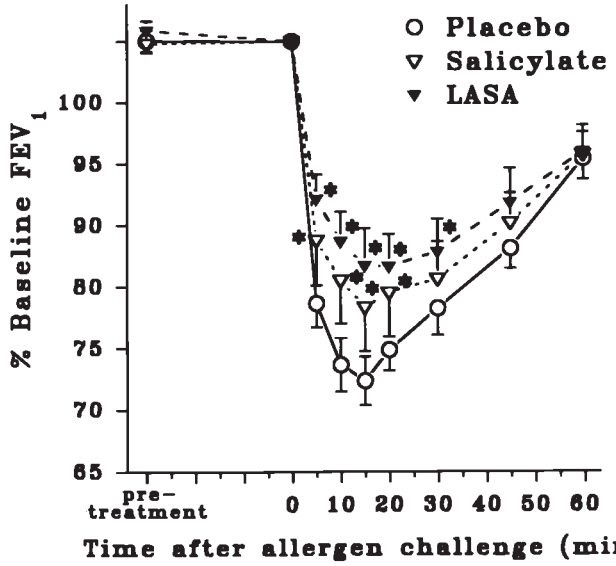

Figure 1. Effect of inhaled lysine acetylsalicylate (LASA) (*solid triangles*), sodium salicylate (*open triangles*), and placebo (*open circles*) on the early asthmatic response. Results are given as the percentage of the FEV_1 value 30 min after treatment, immediately before allergen challenge (time 0). *Bars* represent the mean ± SEM of 12 subjects. *$p < 0.05$ versus placebo at the same time point.

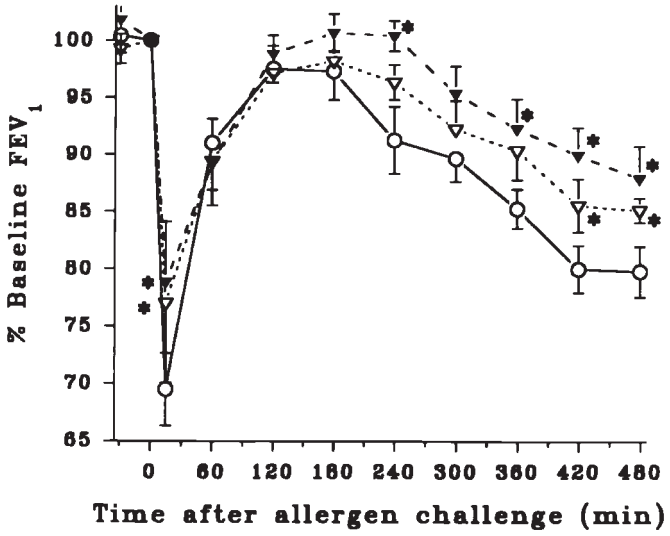

Figure 2. Effect of inhaled LASA (*triangles*), sodium salicylate (*open triangles*), and placebo (*open circles*) on the late asthmatic response. *Bars* represent the mean ± SEM of six subjects. *$p < 0.05$ versus placebo at the same time point.

A p value equal to or lower than 0.05 was considered significant. Unless stated otherwise, data are presented as the mean ± SEM.

RESULTS

Effect of Inhaled Sodium Salicylate and LASA on the Early and Late Asthmatic Reaction to Allergen Challenge

All the treatments were well tolerated and did not cause significant changes of baseline FEV_1 values. The maximum FEV_1 decrease in the first hour after allergen challenge (Figure 1) was 30 ± 2% of baseline after treatment with inhaled placebo, 17 ± 3% after inhaled LASA (corresponding to a degree of protection of 41 ± 9%, $p < 0.005$) and 23 ± 3% after SSA (protection 22 ± 6%, $p < 0.005$). In the six patients presenting a late asthmatic reaction (Figure 2), the maximum FEV_1 decrease between 4 and 8 h after challenge was significantly lower after LASA (13 ± 3%) than after placebo (22 ± 2%), corresponding to a protection of 39 ± 11% ($p < 0.005$). After SSA the maximum decrease in FEV_1 was also significantly reduced compared to placebo (16 ± 2%), with a protection of 23 ± 9% ($p < 0.05$).

When computed from the AUC of the FEV_1 differences from baseline, the protection afforded by LASA and SSA against the early asthmatic reaction (0–60 min) was 31 ± 8% ($p < 0.005$) and 20 ± 9% ($p < 0.05$), respectively. In the six patients presenting a late reaction, protection between 4 and 8 h was 50 ± 19% ($p < 0.025$) after LASA and 34 ± 11% after SSA ($p < 0.025$). All these protection values were significantly different from zero, without significant differences between LASA and SSA.

Effect of Inhaled Indomethacin and LASA on the Early and Late Asthmatic Reaction to Allergen Challenge

All the treatments were well tolerated, although inhaled indomethacin caused irritation of the upper airways and a mild transient bronchoconstriction in some patients. However, this was resolved within minutes and did not cause significant changes of baseline FEV_1 values at the time of allergen challenge. The maximum FEV_1 decrease in the first hour after allergen challenge (Figure 3) was 27 ± 2% of baseline after treatment with inhaled placebo, 18 ± 2% after inhaled LASA (corresponding to a protection of 31 ± 7%, $p < 0.005$) and 29 ± 2% after indomethacin (not significantly different from placebo). In the eight patients presenting a late asthmatic reaction (Figure 4), the maximum FEV_1 decrease between 4 and 8 h after challenge was significantly lower after treatment with LASA (15 ± 2%) and indomethacin (17 ± 4%) than after placebo (25 ± 5%), corresponding to a protection of 39 ± 9%

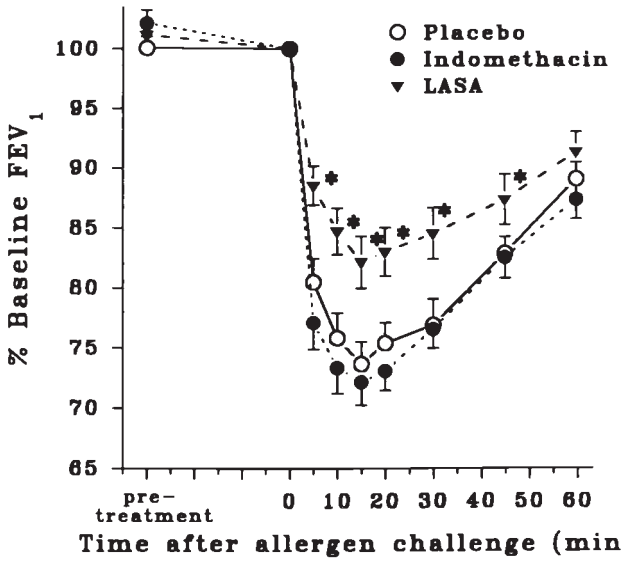

Figure 3. Effect of inhaled LASA (*triangles*), indomethacin (*solid circles*), and placebo (*open circles*) on the early asthmatic response. *Bars* represent the mean ± SEM of 16 subjects. *$p < 0.05$ versus placebo at the same time point.

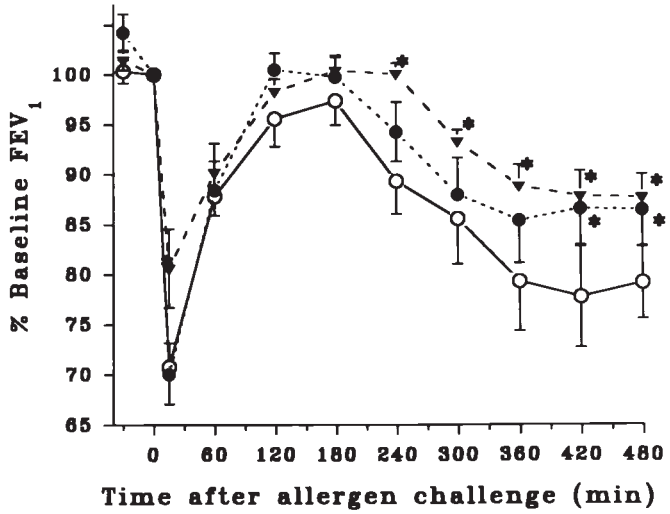

Figure 4. Effect of inhaled LASA (*triangles*), indomethacin (*solid circles*), and placebo (*open circles*) on the late asthmatic response. *Bars* represent the mean ± SEM of eight subjects. *$p < 0.05$ versus placebo at the same time point.

($p < 0.001$) and 30 ± 11% ($p < 0.025$), respectively, without significant differences between LASA and indomethacin.

When computed from the AUC of the FEV_1 differences from baseline, the protection afforded by LASA and indomethacin against the early asthmatic reaction (0–60 min) was 31 ± 8% ($p < 0.005$) and 1 ± 9% (not significant), respectively. In the eight patients presenting a late reaction, protection between 4 and 8 h was 44 ± 18% ($p < 0.05$) after LASA and 39 ± 17% ($p = 0.05$) after indomethacin.

Effect of Inhaled Indomethacin on the Allergen-induced Bronchial Hyperreactivity

Also in this additional group of patients with an early asthmatic response, indomethacin had no effect on baseline FEV_1 nor on the allergen-induced bronchoconstriction (Table 3). However, while after placebo there was a decrease in Mch PD_{20} of 2.0 ± 0.5 doubling doses (with respect to the preliminary pre-challenge test, $p < 0.025$) 2 h after the allergen exposure, after indomethacin the Mch PD_{20} at the same time was unaffected. This protective effect of indomethacin against allergen-induced bronchial hyperreactivity was not due to differences in baseline FEV_1 values among different Mch challenges, since these were similar in the three challenges (Table 4).

DISCUSSION

The results of this study confirm our previous observation that inhaled LASA partially prevents the early and the late asthmatic response to allergen challenge (9), indicating that this drug exerts a protective activity against bronchoconstriction induced by several natural stimuli. However, the activities of indomethacin and SSA against the early asthmatic response were found to be the opposite of their effects against UNW- and metabisulphite-induced bronchoconstriction observed in previous studies (4, 7): inhaled indomethacin was effective against UNW and metabisulphite but had no effect on the early asthmatic reaction, while SSA was ineffective against the former two stimuli but significantly reduced the early response to allergen.

This observation strongly suggests that aspirin-like drugs with different pharmacologic properties may affect different aspects of bronchoconstriction in asthma. If we knew the exact mechanism of action of different aspirin-like drugs, we would be able to provide more information about the differences and similarities between these experimental models of asthma. Unfortunately, the range of the pharmacologic activities of these compounds is not yet fully characterized, although remarkable progress is being made in our knowledge of this field.

The concept that inhibition of cyclooxygenase activity and the suppression of the production of pro-inflammatory prostaglandins is the main mechanism of action of aspirin-like drugs (3) has been confirmed and extended in recent years by the discovery of two different cyclooxygenases, cox-1 and cox-2, variously expressed in different cells and conditions and presenting different sensitivity to inhibition by various aspirin-like drugs (12). Indomethacin and LASA are powerful inhibitors of both cyclooxygenase isoforms although they act through very different mechanisms of action. By contrast, prostaglandin inhibition does not fully explain the anti-inflammatory activity of SSA (13), although this compound can effectively inhibit cox-2–derived prostaglandin production through some unknown indirect mechanism (14). Indeed, the existence of a non–prostaglandin-related mechanism of action of salicylates has been claimed for a long time (15, 16).

Recently a novel mechanism of action has been proposed for aspirin-like drugs based on the inhibition of nuclear factor kappa B (NF-κB) mobilization (17). Salicylates have been

TABLE 4

EFFECT OF INHALED INDOMETHACIN ON ALLERGEN-INDUCED BRONCHIAL HYPERREACTIVITY

	Allergen Challenge				Methacholine Challenge					
	Baseline FEV_1 (1)		%Maximum Fall in FEV_1		Baseline FEV_1 (1)			Mch PD_{20} (μg)		
	Placebo	Indomethacin	Placebo	Indomethacin	Prelim	Placebo	Indom	Prelim	Placebo	Indom
Patient										
1	3.30	3.10	−18.8	−26.2	3.20	3.00	3.20	1800	565	810
2	4.90	4.60	−23.8	−30.4	4.55	4.90	4.55	900	829	900
3	3.85	3.65	−41.8	−28.2	3.65	3.38	3.65	210	91	224
4	2.35	2.30	−30.4	−6.4	2.30	2.15	2.30	2850	190	2600
5	3.85	4.10	−28.6	−22.5	4.00	3.95	4.00	3600	448	1800
6	2.75	2.80	−34.0	−31.5	2.50	2.50	2.50	2700	600	3600
Mean	3.50	3.43	−29.55	−24.19	3.37	3.31	3.37	2010	454*	1656
SEM	0.3	0.35	3.27	3.79	0.36	0.41	0.36	524	112	517

* $p < 0.025$ versus preliminary.

shown to inhibit NF-κB activation, which is required for the production of a variety of inflammatory mediators (18), adhesion molecules (19), cytokines, and nitric oxide (18), by preventing the degradation of the NF-κB inhibitor, IκB, probably through their antioxidant activity. Interestingly this activity is exerted, at relatively high doses, by both ASA and SSA (17) but not by indomethacin (19). Since this pattern of activity is the same as that observed by us for these three drugs against the early asthmatic response to allergen challenge and many of the mechanisms outlined above may be involved in the pathogenesis of asthma (20), it can be speculated that NF-κB activation occurs during allergen-induced reactions and that the protective effect of LASA and SSA against this phenomenon might be mediated by inhibition of this activation, resulting in a blockade of the inflammatory cascade. Further studies are required to clarify whether this or some other unknown mechanism is responsible for the protective effect of LASA and/or salicylate.

Shumitzu and colleagues (10) proposed that the effect of inhaled indomethacin against exercise-induced bronchoconstriction could be due to an inhibitory effect on ion transport similar to that of furosemide. However, this mechanism is unlikely in this case since inhaled furosemide protects against allergen-induced bronchoconstriction (11), while indomethacin had no effect.

Previous studies on UNW-induced bronchoconstriction indicated that aspirin-like drugs may present a different antibronchoconstrictor activity when given by inhalation than when administered systemically (4). When administered orally in a single dose, 4 g of aspirin are required for a significant protective activity against UNW-bronchoconstriction, while inhalation of 500 mg (of which less than 15% would be deposited on the mucosal surfaces) is quite effective. Although there is evidence that salicylates and indomethacin are efficiently adsorbed in the airways (21, 22), there is no doubt that these drugs reach the airway mucosa at concentrations far higher than those attainable after oral administration. Furthermore, there is evidence that local treatment with LASA, at doses far lower than those used in the present study, inhibits cyclooxygenase activity in the bronchi (23). Early studies had suggested that oral indomethacin could reduce the late asthmatic response (24–26), but at least two well-controlled studies eventually indicated a lack of effect of this drug (27, 28). Oral treatment with a cox-2 inhibitor, nimesulide, also failed to affect the response to allergen (29). Since lung cyclooxygenase is known to be relatively resistant to drug inhibition (30), it seems likely that the higher local levels of indomethacin attained by inhalation would account for the protective effect obtained in our study against the late response (4, 10). By contrast, the lack of effect of inhaled indomethacin, at a dose known to affect the bronchial response to other stimuli (4, 7, 10), confirms the conclusions of previous studies *in vivo* (27, 28) and *in vitro* (31) that release of prostaglandins does not play an important role in this phase of the allergic asthmatic response.

Despite having no effect on the early asthmatic response, inhaled indomethacin completely blocked the allergen-induced increase of bronchial responsiveness to Mch occurring 2 h after challenge, as previously observed with inhaled LASA (9). Interestingly, in the study of Kirby and coworkers (27), oral indomethacin was found to inhibit allergen-induced bronchial hyperreactivity measured 24 h after challenge. Although we measured this allergen-induced increase in reactivity in patients with an early asthmatic response (32), this phenomenon is known to be related to the development of the late response (33, 34) and is associated with an influx of inflammatory cells in the bronchial lumen (35). Hence, it is possible that prostaglandins are involved in the development of the late asthmatic response, participating in the pathogenesis of allergen-induced hyperresponsiveness.

In conclusion, our study demonstrates that, when given by inhalation, aspirin, SSA, and indomethacin vary in their ability to protect against allergen-induced early bronchoconstriction in humans but appear to be equally effective against the late response and against allergen-induced hyperreactivity, despite the differences in their mechanism of action. As our knowledge about the mechanism of action of these drugs increases, our findings could be useful for an understanding of the pathogenetic mechanism of bronchoconstriction in asthma and possibly for the identification of new therapeutic strategies for the treatment of this disease.

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