

Rostrum

Report of the INTERASMA Working Group on Standardization of Inhalation Provocation Tests in Aspirin-Induced Asthma

Oral and inhalation provocation tests for the diagnosis of aspirin-induced asthma

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Aspirin (acetylsalicylic acid [ASA]) is the most important of the nonsteroidal anti-inflammatory drugs (NSAIDs). Because of its analgesic, antipyretic, and anti-inflammatory properties, ASA is widely used and sometimes overused.

ASA is a very frequent cause of drug-induced asthma for various reasons: it is widely used, it is available without medical prescription, and it possesses cross-sensitivity with other NSAIDs. Consequently, the number of asthmatics suffering from this type of sensitivity is rather high, and the reactions may be very severe or even life-threatening (1–4).

The first indication of severe untoward reactions to ASA was reported about 100 years ago (5). Some sporadic publications (6–10) followed, but it was only after 1967–8 that studies became more frequent and the

syndrome was characterized. There appear to be several forms of aspirin sensitivity. Reactions include the following:

- 1) asthma associated with rhinitis and conjunctivitis
- 2) urticaria/angioedema in which urticaria appears acutely after ASA ingestion, and chronic urticaria with re-exacerbation after ASA ingestion
- 3) the anaphylactic syndrome in which the skin, gastrointestinal tract, upper and lower airways, and cardiovascular system are affected singly or in combination, with variable severity (11).

Frequently, AIA appears as a combination of three disorders: ASA intolerance, hyperplastic rhinitis with polyps and sinusitis, and severe asthma (the “aspirin triad”) (12).

Estimates of the prevalence of ASA sensitivity vary from 3% to 19%, depending on whether the diagnosis is based on history alone or on challenge tests with ASA, or whether the study population consists of severe hospitalized asthmatics (13). Aspirin-induced asthma affects about 10% of adults with chronic asthma (14–16). The syndrome occurs also in children (17) in whom the incidence is much lower than in adults (18).

Nonsteroidal anti-inflammatory drugs (NSAIDs) cross-react with aspirin and cause a similar acute bronchospasm in aspirin-intolerant asthmatics. NSAIDs are a class of drugs with diverse chemical structures, but which share the property of cyclo-oxygenase (COX) inhibition. The capacity of these agents to provoke asthma is related to their potency as inhibitors of COX (19). The NSAIDs indomethacin and ibuprofen practically always cross-react with aspirin in intolerant individuals. Other NSAIDs cross-react at a somewhat decreased rate (13). Salsalate and acetaminophen are weak inhibitors of COX and cross-react in only a few patients affected by ASA sensitivity and only at a high dose (e.g., acetaminophen 5–6% with 150–600 mg; 28% with 1000 mg) (20).

Mechanism

The mechanism of aspirin-induced asthma has been reviewed by Szczeklik (21), who formulated the COX theory to explain aspirin intolerance (22). According to this theory, asthma attacks are triggered by the specific inhibition in the respiratory tract of the enzyme COX. Szczeklik's observations were confirmed by other investigators (23, 24). It has been suggested that NSAIDs act by shunting arachidonic acid metabolism into the 5-lipoxygenase pathway and by reducing the formation of prostaglandins (PG), which help keep the airways normal, but experimental proof of this is lacking. Perhaps, in AIA, aspirin promotes the release of leukotrienes (LT) by removing PGE₂, a major COX airway product (25, 26).

COX exists in two isoforms, COX1 and COX2 (27). Aspirin, indomethacin, and piroxicam, which at low doses precipitate asthmatic attacks in sensitive patients, are much more potent inhibitors of COX1 than of COX2. In contrast, salicylate, which is tolerated by sensitive patients, has practically no effect on COX. Nimesulide, which is tolerated at a dose of 100 mg, inhibits preferentially COX2. To investigate the effect of COX2 inhibition on aspirin reactions in sensitive individuals, Vaghi et al. (28) subjected ASA-sensitive patients to oral challenge with meloxicam, a relatively specific COX-2 inhibitor. Meloxicam did not induce bronchospastic reactions. The authors concluded that the currently available selective COX-2 inhibitors should be avoided in patients with aspirin-induced asthma. New selective COX2 inhibitors, which are some

1000-fold more potent against COX1, have been synthesized. Perhaps these new selective inhibitors might be useful in AIA (27).

Eosinophils and mast cells play an important role in AIA reactions. Immunohistochemical analysis of nasal polyps of ASA-sensitive asthmatics revealed abundant eosinophils and degranulated mast cells (29, 30). Intranasal challenge with aspirin induced an early influx of eosinophils into the nasal secretions of aspirin-intolerant patients (31). However, serum levels of tryptase, a specific marker of mast-cell activation, increased during aspirin-induced bronchoconstriction (32, 33); this was accompanied by an increase in eosinophil cationic protein and tryptase concentrations. Fifteen minutes after the intrabronchial instillation of 10 mg L-lysine aspirin, there was a significant rise in cys-LTs, IL-5, and eosinophil number in the bronchoalveolar lavage (BAL) fluid of AIA patients, but not of aspirin-tolerant patients (34). Moreover, endobronchial aspirin challenge in ASA subjects caused degranulation of mast cells and eosinophils (35).

The mechanism of NSAID sensitivity involves both modulation of eicosanoid production and increased sensitivity to LTE₄ (36, 37). In fact, challenge of AIA patients with NSAIDs caused significant increases in cys-LT levels in the BAL fluid, nasal secretions, and urine of AIA patients, but not of non-AIA patients or normal subjects (36).

Enhanced release of 15-HETE from the epithelial cells of nasal polyps incubated with lysine-aspirin was observed in ASA-sensitive patients, but not in ASA-tolerant patients (38). The generation of cys-LT is controlled by LTC₄ synthase. This enzyme was dramatically higher in AIA biopsies than in aspirin-tolerant asthmatics or in normal subjects. LTC₄ synthase-positive cell counts correlated significantly with basal levels of cys-LTs in BAL fluid and were associated with enhanced release of cys-LTs in response to endobronchial lysine-aspirin challenge.

Proof of the critical role of LTs came with the advent of anti-LT drugs. Premedication with LT synthesis inhibitors or LT antagonists attenuated nasal and bronchial reactions (39, 40). Moreover, anti-LT agents resulted in bronchodilation, indicating that cys-LTs can affect intrinsic airway tone in AIA (41, 42).

Another mechanism suggested for AIA is that chronic viral infection leads to the production of a population of cytotoxic T cells. Since these cells are usually suppressed by PGE₂ production by alveolar macrophages, COX inhibition may prevent this regulatory mechanism (43). However, there is no evidence to suggest that PGE₂ is suppressed at the doses of aspirin required to elicit a response (44). An element in favor of the hypothesis of viral infection could be the observation that aciclovir, an antiherpesvirus drug, decreases the sensitivity to aspirin in some AIA patients (45).

Diagnosis of aspirin-induced asthma

In patients with severe attacks of asthma in which no other contributing factors can be established, the ingestion of NSAIDs before the event should be thoroughly investigated (46). Ingestion of aspirin or other NSAIDs, apart from anaphylactoid reactions, may provoke either a respiratory response, consisting of bronchospasm, rhinorrhea, conjunctivitis, lacrimation, or urticaria/angioedema. It is rare to observe the respiratory and urticaria responses in the same patient (13, 47, 48).

In normal or presumably normal subjects, the frequency of aspirin intolerance to the oral provocation test (OPT) is extremely low (0.3–0.9%) (21, 49).

Many subjects are unaware of their intolerance to ASA. The fact, for instance, that 15 of 171 (8.8%) of patients with no history or suspicion of aspirin sensitivity had a positive aspirin oral challenge implies that the entity is not suspected by some individuals. On the other hand, about 35% of subjects who were certain they had aspirin sensitivity, had a negative challenge, showing that AIA may be overdiagnosed by history alone (50). In addition, ASA may be included in some compounds considered harmless (i.e., Alka-Seltzer®, pain relievers, and antacids) or in pharmaceutical preparations, sometimes in association with other drugs (codeine, phenacetin and caffeine, propoxyphene, phenobarbital, and ergotamine). The problem is further complicated by the use of NSAIDs in ophthalmic (51) and lotion preparations (52).

Provocation tests

Because the history is not always sufficient and because there is not yet an *in vitro* test for sensitivity to aspirin, the only accurate way to identify the presence of the condition (15) is by oral aspirin or by inhaled lysine-aspirin provocation tests. The main indication for the challenge is the lack of a clear history of aspirin tolerance or a suspected intolerance. But what should be done in patients with a positive history of ASA sensitization? In this context, there is general agreement that the challenge should be done for research purposes or for desensitization. Nevertheless, some authors suggest doing the challenge anyway because there are patients with positive history who can have a negative result in the challenge (50). A negative challenge test in a patient with a positive history cannot exclude ASA sensitivity if the patient is under oral (102) or prolonged treatment with inhaled steroids (77) or has been symptom-free for a long time (78). The inhalation provocation test (IPT) may be negative in patients with urticaria-angioedema syndrome (without respiratory involvement) where the OPT is required (74). Lastly, as a negative nasal provocation test (NPT) does not exclude the possibility of ASA intolerance, the IPT or the OPT is required in these cases.

Methods of provocation

Currently, three methods of provocation are used: the OPT, the IPT, and the NPT. Provocation through the intravenous route has rarely been used with indomethacin (53–55). However, Saga & Suetsugu (54) used the intravenous lysine-aspirin test as a second diagnostic method after the inhalation method. A special method of provocation for research purposes is the local instillation of L-ASA through the bronchoscope (34, 35, 56).

Technical and methodological aspects of provocation

Patients

Patients undergoing provocation tests must be stable and have baseline FEV₁ values of $\geq 60\%$ of predicted and of ≥ 1200 ml. Theophylline and oral β_2 -adrenergics should be stopped 48 h before the test: inhaled β_2 -adrenergic drugs should be stopped 12 h (short-acting) or 24 h (long-acting) before the test. Anticholinergic agents should be stopped 12–24 h before challenge. Sodium cromoglycate and nedocromil sodium should be discontinued 12–24 h before challenge (57).

Corticosteroids can attenuate or prevent bronchospasm induced by a threshold dose even after a 10-day treatment with oral prednisolone (58). Moreover, after long-term treatment (> 6 months) with inhaled steroids, patients with a previous positive response to the L-ASA inhalation test and to the ASA oral test can become negative to both tests (59).

The possibility of false-negative responses increases when diagnostic provocation tests with aspirin are carried out in asthmatics being treated with corticosteroids.

The time interval between ASA challenge and LT drug has not been specifically investigated. Nevertheless, in studies on exercise (60) and cold-air challenge (61), it has been shown that the degree and duration of protection with a LT drug (zafirlukast) were dose-dependent, and that the maximum duration of protection was 12 h. Consequently, it appears advisable to stop anti-LTs 24 h before challenge.

Cautions in provocation tests

The SEPCR “Bronchial Hyperreactivity” Working Group (62) established a set of recommendations and safety procedures to be adopted when performing provocation tests. Full cardiopulmonary resuscitation must be available in the laboratory, and the patient must be under the supervision of a fully trained doctor for 2–3 h. β_2 -Adrenergic stimulants, steroids, vasopressors, intravenous fluids, and oxygen should be readily accessible.

Oral provocation tests (OPT)

OPTs with aspirin are still widely used to diagnose AIA

Table 1. Challenge protocols for 1-day ASA oral test

Authors	Dose increments (mg)						Time interval	Cumulative dosage (mg)
Manning (64)	30	60	100	325	600		Every 2 h	1165
Dahlén et al. (68)	1	10	50	100	300		Every 30–40 min	461
Patriarca et al. (70)	1	10	50	100	500		Every 60 min	661
Christie et al. (44)	30	60	120	300	600		Every 2 h	1120
Picado et al. (3)	10	20	40	80	150	300	Every 30 min	600
Caravaglia (personal communication)	30	60	125	250	500		Every 60 min	965

(47, 63). It should be noted that there are no national or international standard protocols. Two methods are commonly used: the 3-day challenge and the 1-day challenge (69). The 3-day challenge, proposed by Manning & Stevenson (64) is performed in patients with a known history of ASA intolerance. Nevertheless, some investigators prefer (for safety reasons) this type of challenge regardless of the patient's history. The 1-day challenge is used when a clear history of aspirin sensitivity is lacking. Various versions have been described. Dose sequences, the interval between the doses (from 30–45 min to 2 h), and the cumulative doses (from 461 to 1165 mg) differ significantly from investigator to investigator (Table 1).

Spector & Farr (65) divided their patients into four categories according to their history: class I (no history, aspirin ingested within 1 month), class II (no history, no aspirin taken within 1 month), class III (uncertain or suspicious history) and class IV (asthma attack within 24 h: attack after ingestion). They used different graded sequences for each category. Similarly, Caravaglia (personal communication) divided patients into three categories: class I (no history, aspirin ingested in the previous months), class II (uncertain history), and class III (clearly positive history). They administered different graded doses according to the class. A 2-day, single-blinded challenge was used by other authors (66): day 1: placebo, 100 and 200 mg ASA; day 2: 325 and 650 mg ASA. The starting dose varied from 1 to 30 mg (Table 1): a mean starting dose of 10–20 mg was suggested by Szczeklik (67). In addition, there is a wide variability among the protocols of oral ASA challenge. The dosage should probably be tailored according to the characteristics of each patient. In any case, if 650 mg of ASA does not provoke any reaction, the test may be considered negative (11).

Because the OPT can cause serious untoward reactions, it should be performed by a highly experi-

enced doctor in the hospital with resuscitation equipment readily available (68–71). In addition to very severe bronchoconstriction, the OPT can cause severe extrapulmonary reactions (nasal congestion, rhinorrhea, ocular injection, flush, urticaria, or angioedema).

Given these drawbacks, we recommend that the inhalation test be used for the diagnosis of AIA.

Inhalation provocation tests (IPT)

Oral challenge, although still widely used, is time-consuming, and it provokes extrapulmonary symptoms and sometimes severe bronchospastic reactions. Consequently, inhalation challenge with L-ASA is becoming the preferred method. L-ASA is a salt of salicylic acid (lysine acetylsalicylate); it is more soluble than ASA (40% vs 0.3%), is nonirritant, and is well tolerated when inhaled. The original procedure was described by Bianco et al. in 1977 (72). It consisted of inhalation by a jet-nebulizer of increasing doses of L-ASA until a $\geq 20\%$ fall in FEV₁ or a 100% increase in Raw is obtained. Two IPT techniques are currently used:

- 1) continuous inhalation of L-ASA solution at tidal volume breathing from a constant output jet-nebulizer (68, 73, 74)
- 2) intermittent delivery in which a fixed volume of aerosol is delivered to airways during inspiration through metered-dose devices, e.g., the Me-Far dosimeter (75–77) and the Spira Electro-2 dosimeter (78).

Here we describe two L-ASA inhalation methods. In the first method, L-ASA (Flectadol 1000 Maggioni Winthrop, Italy) in the form of a powder containing 1800 mg of lysine acetylsalicylate (equivalent to 1000 mg of aspirin) and 200 mg of glycine is dissolved on each challenge day in 5 ml of distilled water to obtain a lysine aspirin solution containing a 20% concentration of 360 mg/ml (76–78). A volume of 3 ml of this solution is placed in a chamber, and nebulization is achieved through a dosimeter-controlled jet nebulizer (Me-Far MB3 dosimeter, Me-Far Elettromedicali, Brescia, Italy). The delivery time is regulated to produce a nebulization of 5 mg of solution, i.e., 1 mg of L-ASA, for each puff. After inhalation of the diluent control solution, doubling doses of L-ASA, from 1 to 64 mg as

Table 2. Protocol for dosing lysine-aspirin with Me-Far dosimeter (77)

Dosage schedule	Diluent	Drug dosage							
Number of puffs	8								
Single dose (mg)		1	1	2	4	8	16	32	
Cumulative dose (mg)		1	2	4	8	16	32	64	

Increasing doses are given at 30-min intervals.

FEV₁ is measured at 10, 20, and 30 min after each dose.

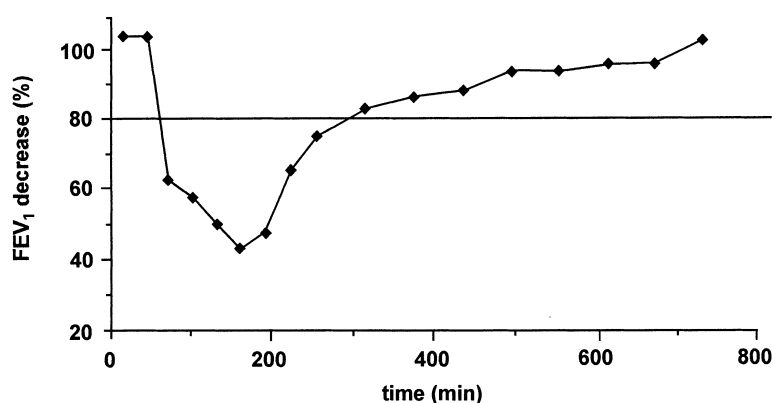


Figure 1. Time course of response to L-ASA inhalation test: "early prolonged reaction" is characterized by slow recovery from induced bronchoconstriction and absence of late reaction.

cumulative doses, corresponding to 0.8 and 51.2 mg of ASA, respectively, are given at 30-min intervals (Table 2).

With the same dosimeter, other researchers administered a cumulative dose of 100 mg of L-ASA, and all positivities were observed with a PD₂₀ of <64 mg (79). The patient, starting at functional residual capacity, slowly and completely inhales the delivered dose, breathing at inspiratory capacity through a mouthpiece and wearing a nose clip. The breath is held for 5 s and then slowly exhaled.

In the second method, L-ASA (Apsol, Bayer, Germany) containing 500 mg of ASA is dissolved in saline (0.9% sodium chloride) (78).

For most sensitive subjects, it is sufficient to prepare two L-ASA solutions (0.1 and 1 M). For less sensitive subjects, a 2 M solution may be necessary. With a 2 M solution of L-ASA and by varying the number of tidal breaths, stepwise increments in the dose of inhaled L-ASA can be achieved to produce the cumulative challenge (Table 3). The nebulization is achieved with the Spire Electro-2 dosimeter.

Challenge procedure

Quantification and follow-up of the reaction. It may be useful to perform a control test with placebo to estimate diurnal variation in lung function and to determine whether the administration of placebo results in a fall of the FEV₁. The most appropriate parameter for the follow-up of a reaction is the FEV₁, which may be associated with the peak expiratory flow rate (PEFR). A fall of $\geq 20\%$ in the FEV₁ is considered a positive response. Baseline FEV₁ is defined as the best of three efforts. If the baseline FEV₁ is above 60% of predicted, the test can be performed unless clinical judgment advises otherwise.

Determination of diluent response and postdiluent baseline. FEV₁ and PEFR are recorded 10 and 20 min after inhalation of the diluent. If stable, these values

constitute the postdiluent baseline. If there is a significant response to the diluent (a deviation of more than 10% from the prediluent value), the diluent inhalation may be repeated. If a significant diluent response persists, the challenge should not be performed that day. In the absence of a diluent response, the provocation can continue, and the 20-min postdiluent baseline is used to calculate the percentage drop in the FEV₁ during the challenge.

Inhalation of lysine-aspirin (78). The L-ASA solution is inhaled every 30 min. The FEV₁ and PEFR are measured at 10, 20, and 30 min after each dose. The provocation is stopped when the FEV₁ has fallen 20% or more from the postdiluent baseline, or the maximum dose of ASA has been reached. If the decrease in FEV₁ at 30 min after an inhaled dose is 15–20%, indicating the development of a positive reaction, it is advisable to wait another 10 min before a further dose increment. If the drop in FEV₁ remains between 15% and 20%, the physician must decide whether or not the next dose in the protocol should be given. In this case, the test should be continued by administering a dose no larger than the last dose.

After a positive reaction, the FEV₁ and PEFR are measured every 15 min until the former returns to within 8–10% of the postdiluent baseline. The patient should always be kept under observation for at least 1 h after

Table 3. Protocol for dosing lysine-aspirin with Spira Electro-2 dosimeter (78)

Aspirin concentration (M)	No. of breaths	Dose (μ mol)	Cumulative dose (μ mol)	Log	Increase in units
0–1	1	1	1	0	0
0–1	2	2	3	1–1	+1
0–1	4	4	7	1–9	+1
1–0	1	10	17	2–8	+1
1–0	4	40	57	4–0	+1
1–0	9	90	147	5–0	+1
2–0	13	260	407	6–0	+1
2–0	35	700	1107	7–0	+1

Table 4. Differences between bronchial and oral provocation tests in aspirin-induced asthma (78)

Drug	Bronchial Lysine-aspirin (L-ASA)	Oral Aspirin (ASA)
Top cumulative provocation dose	64 mg or 1107 μ l (dosimeter method)	Variable (460–1165 mg or higher)
Onset of bronchoconstriction from PD ₂₀	20–45 min	30 min–4 h
Mean FEV ₁ fall	About 30%	About 40%
Drug required for reversal of obstruction	Inhaled β_2 -agonist	Epinephrine, bronchodilators, and corticosteroids
Duration of test	<4 h	>8 h, 3 days
Organ symptoms	Only respiratory	Respiratory and extrarespiratory
Positivity of test	Only in subjects with respiratory syndrome	In all ASA-sensitive subjects (with asthma and with urticaria-angioedema)

termination of the provocation. Although typical late reactions to aspirin challenge have not been documented, the challenged subject should record PEFR in the case of airway symptoms. Before the patient leaves the hospital, the predefined level of drop in PEFR at which rescue medicine should be used, and/or at which the patient should contact the hospital, should be recorded on the PEFR chart.

Patterns of bronchial responses. Phillips et al. (74) and Melillo et al. (76) have studied the time course of the reaction to obtain information on the severity and the recovery of induced bronchoconstriction and the occurrence of late reactions. Bronchoconstriction after L-ASA challenge can appear within 15–30 min, or it may be more gradual. The maximum fall in FEV₁ is reached in 40–90 min. Recovery time from induced bronchoconstriction (return of FEV₁ to within 8% of postdiluent FEV₁) ranges between 2–3 and 6 h or longer. This typical pattern led Melillo et al. (76) to define it as “early prolonged reaction” (Fig. 1). Late reactions have not been observed (68, 74, 76) except by Park (80), who described some doubtful late reactions in his patients.

There are two main differences between the pattern of bronchial responses to L-ASA challenge and that observed after challenge with antigens. One is the long recovery time after induced bronchoconstriction by L-ASA. Although this obstruction develops early, it cannot be classified either as a typical early reaction or as a late reaction. Therefore, it has been designated as the “early prolonged reaction” (76). The second difference is that late asthmatic responses have not been observed after L-ASA challenge.

Bronchoconstriction is reversible by inhaled β -agonists. The test session usually lasts less than 4 h (Table 4). The L-ASA inhalation test is reproducible within a single doubling dose (68–74). A positive provocation response to inhaled L-ASA is followed by a state of refractoriness to further doses of aspirin or other NSAIDs. Complete sensitivity to aspirin and other NSAIDs reappears about 7 days after the last exposure to these drugs. Therefore, repeated challenges should be separated by at least 1 week.

Sensitivity and specificity of IPT compared with clinical history

Phillips et al. (74) have performed the IPT in 11 subjects with a clinical history positive for ASA intolerance. The IPT was positive in all the subjects (sensitivity: 100%). Dahlén & Zetterström (68)(68) examined a group of 19 asthmatics without ASA intolerance. The IPT was negative in all cases (specificity of 100%). Similarly, the sensitivity and specificity of the OPT and IPT are correlated (75).

There was no difference in bronchial hyper-responsiveness (BHR) to methacholine between 23 ASA-sensitive asthmatics and 23 ASA-nonsensitive asthmatics, except for three ASA-sensitive asthmatics who had normal responsiveness (82). This unusual finding may be explained by the fact that these three subjects suffered from episodic asthma with intervals of many months between the attacks. Intermittent asthma can be observed in the initial phases of aspirin-induced asthma, a fact which explains the normal airway responsiveness in the asymptomatic period (82).

In another group of 25 ASA-sensitive asthmatics in which the relationship between nonspecific BHR (PD₂₀ methacholine) and provocation dose to lysine-aspirin (PD₂₀ L-ASA) was examined, there was no correlation between the degree of airway responsiveness to methacholine and L-ASA (78). This result coincides with observations by Phillips et al. (74), who did not find significant correlation between responsiveness to histamine and inhaled L-ASA when expressed as log PC₂₀ values. Even Croce et al. (83) failed to find a significant correlation between the PC₂₀ to histamine and L-ASA. Kowalski et al. (84) were unable to find any relationship between responsiveness to histamine and oral aspirin in 27 aspirin-sensitive subjects. These findings distinguish the L-ASA inhalation challenge from that of allergens in which a correlation between PD₂₀ MT and PD₂₀ allergen has been shown (57).

Of 18 patients, five (27.7%) had a significant decrease in PD₂₀ MT after L-ASA challenge (81). In contrast, Phillips et al. (74) did not find significant changes in airway responsiveness to histamine, expressed as PC₂₀ values, after L-ASA challenge. However, eight of their patients were receiving regular topical steroids, and two

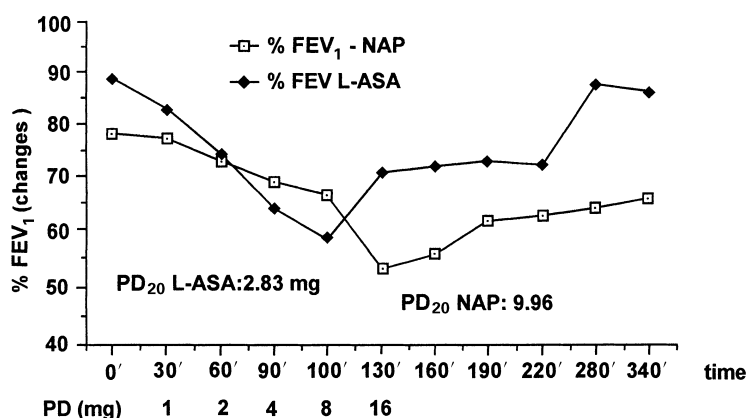


Figure 2. Time course of bronchial response to inhalation tests with naproxen (NAP) and L-ASA in subject with positive history to both NSAIDs.

were receiving oral prednisone, while the patients investigated by Melillo et al. (81) were not taking corticosteroids.

Lastly, BHR was evaluated in a group of ASA-sensitive patients with urticaria and/or angioedema without involvement of the respiratory system. These patients had normal baseline airway responsiveness, negative L-ASA inhalation challenge, and positive oral ASA test. No change was observed in airway responsiveness after both inhaled and oral challenge, even though oral challenge was positive (81). The observation that bronchial responsiveness is normal in subjects suffering exclusively from urticaria or angioedema has been confirmed by others (85).

The presence of BHR in subjects with ASA-induced asthma and its absence in subjects with urticaria and/or angioedema led Melillo (81) to suggest that the presence of BHR or the predisposition to develop it, when ASA is administered, may be considered a definitive marker of distinction between the two subpopulations of ASA-sensitive subjects (those with respiratory symptoms and those with urticaria/angioedema). In other words, ASA-sensitive subjects have an individual sensitivity to aspirin, which is common to all subjects, and an organ-specific sensitivity which distinguishes the two subpopulations. If there is an organ sensitivity at airway level, these patients become hyperresponsive after ASA administration and develop bronchial asthma. If the organ sensitivity is located at skin level, they develop urticaria and/or angioedema.

This hypothesis is supported by the fact that selectivity of pulmonary or cutaneous involvement is mediated by differential expression of lymphocyte-homing receptors (86). On the other hand, Manning & Stevenson (87) have suggested a separate target organ reaction to explain respiratory and cutaneous sensitivity to aspirin.

Six points emerge from these investigations on airway responsiveness in ASA-sensitive subjects:

- 1) The pattern of bronchial responses to L-ASA inhalation tests is different from that observed during provocation tests with antigens or occupational sensitizers. It has a peculiar time course of bronchoconstriction that can be defined as "early prolonged reaction".
- 2) No late reactions have been observed during L-ASA inhalation challenge.
- 3) In some patients with ASA-induced asthma, L-ASA inhalation challenge may induce an increase in airway responsiveness, even in the absence of late asthmatic reaction.
- 4) There is no correlation between nonspecific hyperresponsiveness to methacholine or histamine and the threshold dose of L-ASA.
- 5) In some ASA-sensitive asthmatics who have been free from clinical manifestations for a long time, baseline specific airway responsiveness may be within normal limits, but the hyperresponsiveness appears after inhalation challenge.
- 6) L-ASA inhalation challenges are negative in ASA-sensitive patients with urticaria and/or angioedema.

Prevention of L-ASA-induced bronchoconstriction

Many antihistaminic drugs alleviate aspirin-induced bronchoconstriction and sometimes inhibit extrabronchial symptoms. NSAID-induced bronchoconstriction can be partially inhibited by pretreatment with clemastine, an H₁-receptor antagonist (88), while premedication with terfenadine, a selective H₁-receptor antagonist, was without effect (71). Ketotifen inhibits aspirin-induced bronchoconstriction (89, 90). This drug prevented L-ASA bronchoconstriction in 6/10 patients, and sodium cromoglycate did so in 8/10 patients (91). In another controlled study, cromolyn sodium and nedocromil sodium were equally effective in attenuating aspirin-induced bronchoconstriction (92). Yoshida et al. (93) showed that cromolyn sodium protects against

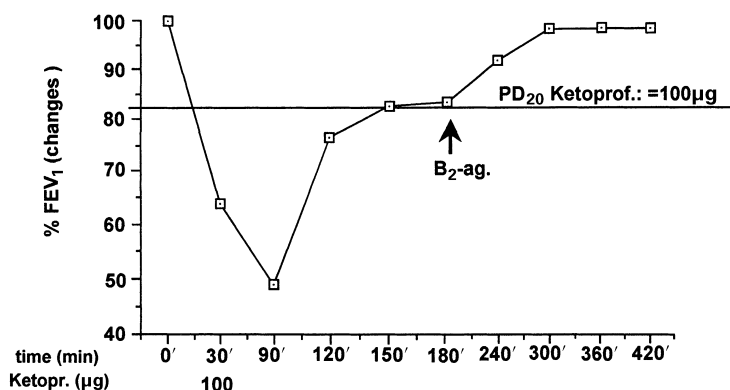


Figure 3. Time course of bronchial response to inhalation test with ketoprofen in subject with positive history to this drug.

sulpyrine-induced bronchoconstriction and prevents urinary LTE_4 excretion in aspirin-induced asthma. Inhaled prostaglandin (PG) E_2 protect against aspirin-induced asthma (94), and inhaled E_2 prevents urinary excretion of LTE_4 after challenge with L-ASA (95, 96).

Inhaled salbutamol and PGE_2 protect against L-ASA-induced bronchoconstriction, and misoprostol, a PGE_1 analog, administered orally 1 h before challenge, attenuates the bronchoconstrictive reactions (97, 98). Salmeterol exerts the same preventive effect (99).

If acutely administered, corticosteroids do not afford protection, but after a 10-day systemic and local pretreatment, there is partial or total protection against aspirin-induced bronchoconstriction (100).

Melillo (81) observed that some patients, positive in the IPT, became negative after prolonged treatment (6 months or more) with inhaled corticosteroid. Interestingly, the same patients were negative also in the OPT.

Inhaled furosemide can block the bronchial response to L-ASA (101) or has a time-limited protective effect against L-ASA-induced bronchial constriction (102).

Various anti-LT drugs inhibit the bronchoconstriction induced in aspirin-intolerant asthmatics by inhaled L-ASA (103, 104) or by oral challenge with aspirin or other NSAIDs (105–107). Zafirlukast (Accolate) was associated with reduced BAL fluid indices of airway inflammation after segmental antigen challenge, suggesting that the cys-LT pathway has potential for modifying the inflammatory response underlying asthma (108, 109). The large body of data supporting the central role of LTs in ASA-induced asthma has led to the introduction of anti-LT drugs as a new treatment for this syndrome (110). Clinical studies have shown that these drugs provide a series of benefits in mild-to-moderate asthma: they may be considered an alternative to other controller drugs, produce an early onset of action, can be used as an alternative to increasing doses of inhaled steroids, and are well accepted by patients so that the treatment has high compliance (107–116). As expected, anti-LT drugs are also beneficial in aspirin-intolerant asthmatics (117, 118).

Anti-LT drugs may provide new strategies for the treatment of asthma and airway inflammation (115), but further clinical trials are needed to determine their precise role in the treatment of asthma (116).

Inhalation provocation tests with NSAIDs other than aspirin

While L-ASA inhalation tests are used in many laboratories and are being adopted by an increasing number of investigators, inhalation tests with other NSAIDs have been performed only episodically, except in Japan, where L-ASA was not easily obtained on the local market. The drug first used in Japan is sulpyrine (noramidopyrine) (121, 122). Suetsugu et al., who have wide experience in this field, suggest the following protocol with increasing concentrations of sulpyrine: 1:0–2:0.1–3:1–4:10–5:25%. The interval between doses was 30 min. The mean PC_{20} in these cases was 3.1%; sensitivity was 90% and specificity 85%. The test produces quick responses and rapid recovery from the bronchoconstriction, which is mild compared to that observed in pyrazolone sensitivity (123). Other drugs used in Japan by the inhalation route are tolmetin (an indole derivative) (123) and dypirone (124).

Martelli has performed the inhalation test with indomethacin (53). Melillo et al. (125) have performed it with noramidopyrine (NOR), naproxene (NAP), and L-ASA in a 67-year-old woman who had a history of chronic bronchitis, intermittent asthma, and sensitivity to the three drugs. The time course of the response with the three drugs was compared. The response to NOR was characterized by a late appearance of the bronchoconstriction, a high provocation dose (PD_{20} : 85.1 mg), and a slow recovery from the induced bronchoconstriction. The provocation doses (PD_{20}) during the L-ASA and NAP tests were 2.83, and 9.96 mg, respectively. The recovery from induced bronchoconstriction was more rapid during the L-ASA test than during the NAP test (Fig. 2).

Melillo et al. (personal communication) have performed the inhalation test with another NSAID:

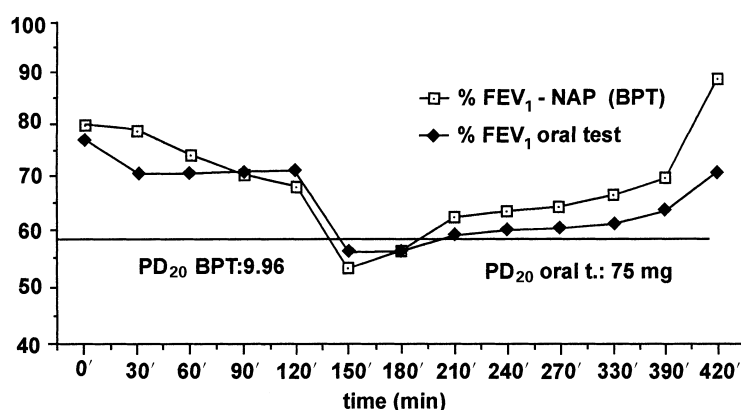


Figure 4. Time course of bronchial response to inhalation and to oral tests with naproxen (NAP) in subject with positive history to this drug.

ketoprofen. The bronchial response to the test was of the "early prolonged reaction" type (Fig. 3), as observed during the IPT with L-ASA.

It is noteworthy that there was a significant difference in the provocation doses between the various NSAIDs and NOR, probably in relation to the different potency as inhibitors of COX of NSAIDs and analgesics.

Finally, oral and inhalation tests with NAP were performed in one patient in order to study the characteristics of the response to the two tests. The time course of the responses appeared to be similar (Fig. 4). However, there were two main differences:

- 1) The PD_{20} was 9.96 mg in the bronchial test and 75 mg in the oral test, confirming an advantage of the inhalation test, which requires a significantly lower dose of drug than the oral test.
- 2) The recovery from induced bronchoconstriction was more rapid after the inhalation test than after the oral test.

In conclusion, NSAIDs other than aspirin can be used for inhalation provocation tests, but the practical application in the clinical setting requires further investigation.

Aspirin desensitization

Aspirin desensitization in its broadest sense refers to reducing the reactions to ASA by repeated and increasing exposure to ASA until all reactions cease (126). Aspirin desensitization may be achieved by oral routes or by topical (intranasal and intrabronchial) routes. The phenomenon of desensitization was observed, among the first, by Bianco et al. (127). They obtained the desensitization of six ASA-sensitive asthmatic patients by administering increasing doses of lysine-aspirin by inhalation, and then increasing the dose of ASA by mouth. At the end of the trial (within 20 days), all patients tolerated perfectly 500 mg of ASA by mouth. Oral desensitization to aspirin can be induced, in the Stevenson protocol (128), by giving incremental

doses of aspirin in 5 days until the well-tolerated dose of 650 mg is reached. The desensitized state persists for 2–5 days, with full sensitivity returning after 7 days. ASA can be administered daily, with doses of 80–325 mg to maintain desensitization (129). If treatment is administered in daily doses of ASA (650 mg b.i.d.), patients usually experience improvement in their underlying chronic respiratory symptoms, especially in the nose, during the maintenance of the desensitized state for months or even years.

The phenomenon of cross-desensitization is achievable also for the NSAIDs that cross-react with ASA (130). Patriarca et al. (131) and Patriarca (132) have induced sensitization by the nasal route and have used the topical application of L-ASA for the prevention of relapse in nasal polyposis present in the aspirin triad.

The phenomenon was confirmed in a study in which the L-ASA inhalation technique was used to obtain desensitization (133). Two procedures were used to obtain desensitization, which was called "adaptive disactivation" to distinguish it from the desensitization obtained by specific immunotherapy in allergic asthma. The first procedure, called "rush disactivation", is obtained in 2 days by administering L-ASA until induction of tolerance. The second procedure, called "individual dose titration", is a combination of inhalation and oral administration of ASA, and is more time-consuming, and complete tolerance (which means that a single dose of 500 mg of ASA is safety tolerated) is generally induced in 12 days.

Nasal provocation tests (NPT)

NPTs have been used less frequently for research (134–136) or for the assessment and diagnosis of ASA (136). Of seven patients with aspirin-induced asthma, diagnosed from inhalation challenge and subjected to nasal challenge at least a week later, only two severe cases had a positive nasal response during challenge (significant changes in nasal airway resistance, rhinomanometry, and sneezing), while all seven patients had a

decrease in FEV₁ of 15% or more (15–31%) (137). The authors suggest that the NPT may be suitable for diagnosing ASA-induced asthma.

More recently, Milewski et al. (138) examined the NPT with L-ASA as an alternative technique for detecting aspirin-induced asthma. Unlike other investigators who used L-ASA inhalation in the nostrils, Milewski et al. instilled L-ASA droplets locally from a micropipette on the lower nasal concha in both nostrils. Nasal flow measurements were made with anterior rhinomanometry at 10, 20, and 30 min and by scoring the subjective nasal and extranasal symptoms of aspirin intolerance. Threshold doses of L-ASA for the NPT were 20-fold lower than those for bronchial challenge, and 1000-fold lower than those for oral provocation. There were no systemic reactions. The test was highly sensitive (86.7%) in a group of patients with AIA confirmed by oral challenge, and specific (95.7%) in a group of patients with negative oral challenge. However, the negative result does not exclude the possibility of aspirin intolerance (value excluding diagnosis: 78.6%). The authors suggest the use of the NPT in patients with instability of the bronchial tree or poor lung function in whom bronchial or oral provocation tests with ASA are contraindicated.

Patients suspected of aspirin intolerance with negative results in the NPT should undergo bronchial or oral challenge tests with aspirin. In conclusion, the NPT can

be used as a procedure in outpatients, but its value is limited by lower sensitivity than oral or inhalation tests (67).

Conclusions

Many elements distinguish OPT with ASA from IPT with L-ASA (Table 4). The IPT is less time-consuming and usually does not provoke severe bronchoconstriction. The provocation doses in the IPT are significantly lower than in the OPT. The induced bronchoconstriction is rapidly reversed by an inhaled β_2 -agonist (78).

A concentration or dose-response curve can be constructed with the IPT, which also gives a quantitative estimation of airway responsiveness to the drug. A crucial difference between the two methods is that IPT does not cause extrarespiratory reactions, while the OPT can stimulate severe reactions and may sometimes require intensive pharmacologic treatment (epinephrine, bronchodilators, and corticosteroids).

Given its many advantages, the IPT will probably replace oral challenge in the detection of ASA-induced asthma, while the OPT is required for ASA-induced urticaria and/or angioedema.

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