Original paper

Extrabronchial symptoms and late phase reaction enhance the diagnostic value of aspirin bronchial challenge

Damian Tworek[1], Ewa Zielińska-Wyderkiewicz[2], Paweł Górski[2], Piotr Kuna[1]

[1]Department of Internal Diseases, Asthma and Allergy, Barlicki University Hospital, Medical University of Lodz, Lodz, Poland
Head of the Department: Prof. Piotr Kuna MD, PhD
[2]Department of Pneumonology and Allergy, Barlicki University Hospital, Medical University of Lodz, Lodz, Poland
Head of the Department: Prof. Paweł Górski MD, PhD

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Abstract

Introduction: Lysine aspirin (l-ASA) bronchial challenge can be used in the diagnostics of aspirin exacerbated respiratory disease. It is safer than oral challenge, however it is characterized by a lower sensitivity.
Aim: We sought to investigate whether additional indicators of the positive result of l-ASA bronchial challenge, i.e. late phase reaction (LPR) and extrabronchial symptoms (EBS), may enhance its diagnostic value.
Material and methods: Sixty-seven patients with a positive history of asthma exacerbated by aspirin and/or other non-steroidal inflammatory drugs underwent l-ASA bronchial challenge. The control groups comprised 15 aspirin tolerant asthmatics and 15 healthy subjects. Forced expiratory volume in 1 s (FEV_1) and 24-hour peak expiratory flow (PEF) measurements were performed in all subjects in order to recognize early and late response to l-ASA. All subjects underwent oral ASA challenge 2 weeks after l-ASA bronchial challenge.
Results: Basing on FEV_1 and PEF results, early reaction was present in 50.7% of patients, early and LPR in 29.9% and LPR in only 10.4% of aspirin exacerbated respiratory disease patients. The EBS were noted in 31.3% of subjects. Inclusion of LPR and EBS as positive criteria of the challenge increased sensitivity to 94.0%.
Conclusions: These results indicate that both LPR and EBS should be considered as positive criteria of aspirin bronchial challenge as they enhance its diagnostic value.

Key words: aspirin, late phase reaction, extrabronchial symptoms.

Introduction

The prevalence of aspirin exacerbated respiratory disease (AERD, formerly named aspirin induced asthma) among asthmatics ranges from 4.3% in Poland to 8.8% in Finland and 10.9% in Australia [1–3]. It has been shown that aspirin hypersensitivity may be one of the factors responsible for development of severe asthma phenotype [4]. So far, no reliable *in vitro* test has been developed and therefore, in subjects suspected of aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) intolerance, aspirin provocation should be carried out for correct diagnosis and treatment. In order to diagnose AERD, three types of challenge can be used: 1) oral, single-blind placebo controlled challenge with aspirin, 2) bronchial and 3) nasal-single blind, placebo controlled challenges with lysine aspirin (l-ASA) [5]. L-ASA bronchial challenge was introduced as a diagnostic procedure by Bianco *et al.* and its usefulness was subsequently proven by others [6–8]. Some authors reported presence of late phase reaction (LPR) after l-ASA bronchial challenge, indicated by bronchoconstriction occurring several hours after the provocation test [9]. Others examined the effect of inclusion of extrabronchial symptoms (EBS) in the diagnostic value of aspirin provocation test [10]. To our knowledge, no study has been carried out to assess the diagnostic value of bronchial l-ASA challenge where both LPR and EBS were considered as additional criteria of a positive challenge results.

Aim

Therefore, the aim of our study was to determine the clinical pattern of reactions after l-ASA bronchial challenge and evaluate the diagnostic value of this method with regard to early phase reaction (EPR) and LPR as well as EBS, in patients with aspirin induced asthma.

Address for correspondence: Damian Tworek MD, Department of Internal Diseases, Asthma and Allergy, Barlicki University Hospital, 22 Kopcinskiego St, 90-153 Lodz, Poland, phone: +48 42 677 69 39, fax: +48 42 679 03 16, e-mail: damian.tworek@gmail.com
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Material and methods

Subjects

Subjects participating in this study were divided into 3 groups. Patients with mild to moderate asthma and hypersensitivity to NSAID confirmed by a positive result of oral ASA challenge were included in the aspirin intolerant asthmatics group (AIA). The second group, aspirin tolerant asthmatics (ATA), comprised subjects with mild to moderate asthma and negative oral ASA challenge. The third group consisted of healthy controls, with no history of asthma and negative results of oral ASA challenge (ASA tolerant non-asthmatics, ATNA). ASA oral challenge was performed 2 weeks after the bronchial challenge. All subjects gave their informed consent and the study protocol was accepted by the Ethical Committee of Medical University of Lodz.

Lysine ASA solutions

ASA bronchial challenge was performed with crystalline l-ASA (Aspisol, Bayer, Leverkusen, Germany) using a dosimeter-controlled jet nebulizer (Spira Electro 2, Respiratory Care Centre Hämeenlinna, Finland) with maximal inspiratory flow 0.5 l/s, inspiratory volume 0.5–0.6 l, single inspiration time 0.8 s and 10.3 µl of l-ASA solution per inspiration. L-ASA solutions were prepared on the day of the challenge by dilution of lys-ASA in 0.9% NaCl, and in each case two concentrations were prepared: 180 mg/ml (1.0 M) and 18 mg/ml (0.1 M). The maximum cumulative dose of l-ASA was 97.2 mg. Solutions were stored at –2°C to –8°C and thawed in room temperature for 10 min before use.

Lysine ASA inhalation challenge

Single-blind, placebo controlled l-ASA bronchial challenge was performed during 7 consecutive days. Patients withdrew oral and nasal steroids, antileukotrienes and antihistamines 28 days prior to the study; cromones 7 days prior to the study; inhaled steroids, long acting theophylline preparations and long acting β_2-agonists 24 h prior to the study; short acting β_2-agonists 8 h before the challenge. None of the participants had been treated with omalizumab or any other biological agent.

On the first day, patients received 30 inhalations of 0.9% NaCl, which was repeated 3 times at 30-minute intervals. Forced expiratory volume in 1 s (FEV_1) was measured 10 and 20 min after each series of inhalations. Afterwards, the patients filled in self-observation diaries and performed peak expiratory flow (PEF) measurements.

On the third day, placebo – lysine solution with acetic acid, which had a pH and osmolarity similar to l-ASA was consecutively administered in the following manner: 1, 3, 7, 10, 20 and 30 inhalations. The FEV_1 was measured 10 and 20 min after each series of inhalations. Following this, patients filled in self-observation diaries and performed PEF measurements over 24 h.

On the fifth day, l-ASA was administered. After basal spirometry, the challenge was started with 7 inhalations of 0.9% NaCl, spirometry was performed 10 and 20 min after the last inhalation. If the FEV_1 value fell below the baseline FEV_1 by more than 10%, the procedure was ended. The PEF measurements were performed and the symptoms diary was filled in by patients after the challenge over the following 24 h. L-ASA administration sequence is shown in Table 1.

A reduction of 20% or more in FEV_1 compared to the post saline value was considered a positive challenge.

Oral ASA challenge

All subjects underwent single-blind, placebo controlled oral challenge with ASA. The challenge was carried out according to the protocol used at our department. On the first day, patients received placebo (saccharin lactate in gelatin capsules) which on the second day was followed by increasing doses of ASA administered at 1-hour intervals (10, 20, 40, 80, 120, 160, 300, 600 mg). The FEV_1 was measured every 30 min. A decrease in FEV_1 of 20%

Table 1. Lysine aspirin bronchial (l-ASA) challenge protocol

L-ASA concentration [mol/l]	No. of inhalations	Administered dose [µmol]	Cumulative dose [µmol]	Cumulative dose [mg]
0.1	1	1	1	0.18
0.1	2	2	3	0.54
0.1	7	7	10	1.8
1	2	20	30	5.4
1	7	70	100	18
1	8	80	180	32.4
1	12	120	300	54
1	24	240	540	97.2

or more was considered as a positive result of the challenge.

Atopic status

Patients were considered as atopic when at least one positive skin prick test result (wheal diameter ≥ 3 mm) for common aeroallergens was present. The aeroallergens tested were Dermatophagoides pteronyssinus, Dermatophagoides farinae, grasses, birch, hazel, alder, mugwort, cat, dog, Alternaria tenuis and Cladosporium herbarum (Allergopharma, Reinbeck, Germany).

Spirometry and PEF measurements

The FEV_1 and forced vital capacity (FVC) measurements were performed using Spirometer Lung Test 1000 (MES, Krakow, Poland) according to ATS/ERS guidelines at the time points specified above [11]. The PEF was measured by patients under supervision of a physician or qualified nurse every 60 min, over 24 h after the challenge using Mini-Wright device (Clement Clark International, Harlow, England). A decrease in PEF of 20% compared to PEF value measured before the challenge was considered as a late reduction in PEF and an indicator of LPR.

Statistical analysis

Statistical evaluation was carried out using Statistica 8.0 software (StatSoft, Tulusa, OK, USA). Descriptive statistics were expressed as mean ± standard deviation if not stated otherwise. Shapiro-Wilk test was used for testing the normality of distribution. *t*-Student test and *U*-Mann-Whitney test were used to compare parametric and non-parametric variables, respectively. The χ^2 test or Fisher exact test were used, when applicable, to compare frequencies. Kruskal-Wallis test was used to compare several groups. Relationships between variables were studied with Spearman's *R* correlation coefficient. A p value < 0.05 was considered statistically significant.

Results

ATA subjects and healthy controls

Characteristics of all subjects are presented in Table 2. Neither a 20% or greater fall in FEV_1 nor a late reduction in PEF and EBS were observed in ATA subjects and healthy controls after l-ASA bronchial challenge.

AIA Subjects

Based on changes in FEV_1 during the provocation and changes in PEF during the following 24-hour PEF monitoring period, we distinguished 3 groups of AIA patients:

Group I – early phase reaction only

A minimum 20% fall in FEV_1 during aspirin bronchial challenge occurred in 34 (50.7%) AIA patients, mean age 49.42 ±12.98 years. No significant fall in PEF values during the 24-hour period of PEF observation was noted. The mean value of PD_{20} was 2.47 mg (range: 0.15–4.86 mg).

Group II – early and late phase reaction

Twenty (29.9%) AIA subjects, mean age 44.63 ±7.97 years had early, as well as late phase reaction after aspirin inhalation challenge characterized by at least a 20% fall in FEV_1 during the provocation and a significant decrease in PEF within several hours (4–11 h) after the challenge. The mean value of PD_{20} was 2.04 mg (range: 0.06–3.40 mg).

Group III – late reaction only

A response limited only to late reaction was observed in 7 (10.4%) patients, mean age 38.14 ±8.51 years. None of these had a 20% fall in FEV_1 or bronchial and extrabronchial clinical symptoms during aspirin challenge. However, a significant fall in PEF was noted between the 4^{th} and 11^{th} hour after the end of aspirin provocation.

In 6 (9.0%) AIA patients, neither a 20% or greater fall in FEV_1 nor a late reduction in PEF were observed.

Comparison of the groups

No significant differences in gender distribution were observed (p = 0.42), patient age (p = 0.14) and l-ASA PD20 (p = 0.91) between groups I and II. Patients from group III were significantly younger than patients from group I + II (p = 0.037). Group I + II and group III did not differ significantly with respect to gender distribution (p = 0.11)

Although patients from group I tended to be older when compared to group II + III, the difference was not

Table 2. Patients' characteristics

Parameter	AIA (n = 67)	ATA (n = 15)	Healthy controls (n = 15)
Age [years]	46.7 ±11.69	39.4 ±17.07	39.27 ±14.83*
Patients, *n* (%)	48 (71.6)	10 (66.7)	9 (60)
Atopy, *n* (%)	41 (61.2)	10 (66.7)	0 (0)
Baseline FEV_1 % of predicted	83.16 ±11.69	97.53 ±12.81	98.53 ±11.51#

*AIA – aspirin intolerant asthmatics, ATA – aspirin tolerant asthmatics. *AIA vs. ATA vs. healthy controls, p = 0.0442; #AIA vs. ATA vs. healthy controls, p = 0.0003.*

significant (48.27 ±13.15 and 42.85 ±8.19, respectively, $p = 0.064$). No difference was found in sex distribution between these groups ($p = 0.19$).

Extrabronchial symptoms during lys-ASA challenge

In addition to monitoring spirometry results, clinical bronchial and extrabronchial symptoms of aspirin sensitivity were also recorded during the challenge. Bronchial symptoms (dyspnea, chest tightness, wheezing, cough) were clinical manifestations of decreased airflow. Extrabronchial symptoms were observed in 18 patients (11 women and 7 men) and they often preceded bronchoconstriction. Extrabronchial symptoms were noted in 2 AIA patients without ≥ 20% fall in FEV_1 after l-ASA bronchial challenge. The median of cumulative dose that resulted in extrabronchial symptoms was 65 µmol (25 percentile: 30 µmol; 75 percentile: 180 µmol) and median of cumulative dose that provoked bronchial symptoms was 240 µmol (25 percentile: 100 µmol; 75 percentile: 540 µmol). The difference was statistically significant ($p = 0.016$).

A significant association between atopic status and presence of EBS was observed ($p = 0.034$).

Extrabronchial symptoms were also noted in 3 of 7 patients with late reaction after l-ASA challenge (Table 3).

The most frequent extrabronchial symptoms, affecting 7 (38.8%) patients, were nasal symptoms (rhinorrhea, nasal congestion). Detailed characteristics of EBS are shown in Table 4.

Diagnostic value of l-ASA bronchial challenge with respect to early and late reaction and extrabronchial symptoms

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of l-ASA bronchial challenge with regard to the different criteria of positive challenge are presented in Table 5. The best diagnostic value was achieved when all of the positive criteria of l-ASA challenge (min. 20% fall in FEV_1, late reduction in PEF and EBS) were taken into consideration.

Discussion

During and after the provocation test we observed EBS in one third of AIA patients, the most frequent of which were nasal symptoms. Presence of EBS during ASA provocation tests has been previously reported [8, 10, 12]. Dahlen *et al.* [8] noticed that generalized symptoms were more common during oral than bronchial challenge. Niżankowska *et al.* [10] analyzed the occurrence of EBS in 35 AIA patients undergoing oral ASA and bronchial l-ASA challenges. Oral provocation test was positive in 24 subjects when only a min 20% fall in FEV_1 was used as a criterion of positive challenge (sensitivity 69%, specificity 100%). When the authors considered the occurrence of EBS as a positive test result, the positivity increased to 31 patients (sensitivity 89%, specificity 93%). The same pattern was seen during l-ASA bronchial challenge. Positivity increased from 21 patients when measured as a min 20% fall in FEV_1 only (sensitivity 60%, specificity 100%), to 27 subjects when EBS were taken into account (sensitivity 77%, specificity 93%).

In this study, sensitivity was somewhat higher and reached 80.5% when positivity was measured only on the basis of spirometry criteria and 83.6% when in addition EBS were considered as a positive endpoint. The specificity remained unchanged at a high level of 100%, as neither a fall in FEV_1 nor any EBS in ATA and healthy subjects was observed.

Patients undergoing l-ASA bronchial challenge were monitored over 24 h after the test was ended. In 27 subjects LPR was noted, of these 20 patients had a min 20% fall in FEV_1 during the provocation. However, in 7 cases neither clinical symptoms nor a fall in FEV_1 occurred. There is some controversy in the literature regarding the presence of LPR. Phillips *et al.* [7] denied the occurrence of LPR after l-ASA bronchial provocation. In 11 test subjects, bronchoconstriction occurred during the challenge but none of them exhibited a fall in FEV_1 during the 8-hour period of observation following the challenge. Melillo *et al.* [13] did not observe definite symptoms of LPR, but described a condition that they referred to as

Table 3. Bronchial and extrabronchial symptoms in patients with late reaction only (a significant fall in PEF within 4 to 11 h after l-ASA challenge)

No.	Age [years]	Gender	Extrabronchial symptoms (onset after the end of the challenge)	Bronchial symptoms (onset after the end of the challenge)
1	39	F	None	Dyspnea (5 h)
2	39	F	None	Dyspnea (8 h)
3	40	F	Rhinorrhea (6 h)	None
4	22	F	None	Dyspnea (5 h)
5	49	F	Angioedema (7 h)	Dyspnea (7 h)
6	44	F	Throat itching (4 h)	None
7	34	F	None	Dyspnea (8 h)

Table 4. Bronchial and extrabronchial symptoms during l-ASA challenge

No.	Age [years]	Gender	L-ASA cumulative dose [µmol]/ extrabronchial symptoms	L-ASA cumulative dose [µmol]/ bronchial symptoms
1	73	F	300/urticaria, generalized pruritus	540/dyspnea
2	48	F	30/urticaria, dermal flush	180/dyspnea
3	28	M	180/erythema	300/dyspnea
4	60	F	180/generalized pruritus	300/dyspnea
5	66	F	30/rhinorrhea	180/dyspnea
6	49	F	100/throat itching	300/dyspnea
7	36	F	10/rhinorrhea	30/dyspnea
8	62	M	300/eye redness and itching	540/dyspnea
9	44	F	240/generalized pruritus, urticaria	300/dyspnea
10	40	F	30/rhinorrhea	30/dyspnea
11	42	M	30/dermal flush	100/dyspnea
12	23	M	100/dermal flush	100/dyspnea
13	47	M	30/rhinorrhea, nasal congestion	540/dyspnea
14	60	M	30/rhinorrhea	540/dyspnea
15	38	F	100/rhinorrhea	180/dyspnea
16	40	F	1/rhinorrhea	10/dyspnea
17*	54	F	540/generalized pruritus	None
18*	25	M	540/dermal flush	None

**Patients with extrabronchial symptoms but without ≥ 20% fall in FEV_1 after l-ASA inhalation.*

Table 5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of l-ASA bronchial challenge depending on different criteria of the positive result of the challenge

Parameter	Fall in $FEV_1 \geq 20\%$ (%)	Fall in $FEV_1 \geq 20\%$ and late fall in PEF (%)	Fall in $FEV_1 \geq 20\%$ and EBS (%)	Fall in $FEV_1 \geq 20\%$ and EBS and late fall in PEF (%)
Sensitivity	80.5	91	83.6	94.0
Specificity	100	100	100	100
PPV	100	100	100	100
NPV	69.8	83.3	81.1	88.2

"early prolonged reaction". This was characterized by an early fall in FEV_1 followed by slow, spontaneous recovery within several hours. Clear LPR after l-ASA bronchial challenge was first described by Park [9]. He observed this in several patients with a history of ASA hypersensitivity and dual response after bronchial challenge – early fall in FEV_1 was followed by recovery and subsequent late bronchoconstriction after 4 to 7 h.

In this study, a late fall in PEF was the most important indicator of LPR. When this criterion was taken into account, sensitivity reached 91%, whilst specificity remained unchanged as there was no late fall in PEF in patients without ASA hypersensitivity.

The results of our study suggest that both LPR and EBS should be considered as positive results of l-ASA bronchial challenge, which could enhance the diagnostic value of the test.

Conflict of interest

The authors declare no conflict of interest.

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