ORIGINAL CONTRIBUTION

Rhinology, 48, 216-223, 2010

Evaluation of nasal mucosal swelling and microcirculation throughout nasal and bronchial provocation tests with lysine-aspirin in asthmatics with nasal polyposis*

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SUMMARY

According to the GA2LEN recommendations, nasal challenge test with lysine-aspirin should be performed only in patients with severe asthma, because the sensitivity of this test has been lower than in bronchial and oral challenge tests. The AIA patient group often have severe asthma with impaired lung function, and therefore improvement of the nasal challenge is warranted. The outcomes of this study clearly indicate that a prolonged detection time from two to three hours might improve the sensitivity of the nasal challenge as a method for diagnosing aspirin intolerance. Moreover, we found a different vascular response in the nasal mucosa in the subjects with AIA after local challenge with lysine-aspirin as compared to an ATA patient group. This puts RSM-LDF as a possible new method in addition to those previously recommended for this particular test.

Key words: aspirin intolerance, asthma, nasal polyposis, lysine-aspirin, challenge test, laser Doppler flowmetry, rhinostereometry, pnif, broncho-nasal reflex

INTRODUCTION

Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) are common reasons for adverse drug reactions, and were reported to be the second to antibiotics as the most frequent agents causing drug hypersensitivity [(1)], and previously reported to cause 21-25% of all adverse reactions [(2)]. The diagnosis is based on a typical history, confirmed by a positive aspirin provocation test [(3)]. The prevalence of aspirin intolerance (AI) in the general population has been estimated to be between 0.6%-2.5% [(4)], and the prevalence of nasal polyposis in adults with a diagnosis of AI is estimated to 36% [(5)]. AI is especially common in patients with asthma, and the frequency of AI among adult asthmatics (AIA) was reported to be about 5% when the diagnosis was relying on history alone, and when based on provocation tests coupled with spirometry, it was 8-20% [(6)]. This underlines the importance of using provocation tests when diagnosing AI.

Three different challenge methods are available today to detect AI. The oral challenge test was the first one developed in the 1970s and has been used in clinical practice [(7)]. The sensitivity and specificity of the method is high, but it is time-consuming and there is always a risk of producing severe bronchial and systemic reactions [(8)]. According to GA2LEN guidelines, the oral aspirin challenge should only be used for the diagnosis of AIA (aspirin-intolerant asthma), AIA/R (aspirin-intolerant asthma and rhinosinusitis) and AIU (aspirin-induced urticaria/angioedaema) in experienced medical centres [(3)]. Bronchial challenge with the inhalation of increasing doses of lysine-aspirin and the detection of the bronchial response with spirometry is better tolerated, as the symptoms are mainly located in the bronchi and sometimes in the nose [(8,9)]. However, as with all inhalation challenges, lysine aspirin is not recommended in patients with low lung function or unstable asthma [(3)].

Nasal challenge tests with lysine-aspirin have therefore been developed as a safer and simpler alternative, and over the last ten to twenty years several studies describing nasal challenge with lysine-aspirin have been published [(10-12)]. Furthermore, it has been proposed that the nasal lysine-aspirin challenge can be performed in outpatient settings [(3)]. GA2LEN recommends that when considering a nasal aspirin challenge, the nasal cavity has to be examined and excessive nasal polyposis and septal

*Received for publication: May 26, 2009; accepted: August 28, 2009

DOI:10.4193/Rhin09.090

deviation have to be excluded [3]. Four different methods have been used as challenge assessments: clinical symptoms, acoustic rhinometry, active anterior rhinomanometry and peak nasal inspiratory flow (PNIF) [3]. As a new method, the detection of urinary LTE4 after nasal challenge has recently been proposed [13].

The aim of this study was to investigate the nasal reaction time course after a single spray dose of lysine-aspirin for 180 minutes to examine possible late reactivity in the nasal mucosa. In addition, we wanted to evaluate the vascular response of the nasal mucosa after nasal challenge with lysine-aspirin, and detect possible differences between AIA and ATA patient groups. To study events in both the deep and the superficial part of the nasal mucosa, we used rhinostereometry with a connected laser Doppler flowmetry apparatus, a method used in different histamine challenge tests [14,15]. We intended to relate this method to PNIF and symptom scores, which are already established methods. Finally, we wanted to evaluate a possible reaction in the nasal mucosa upon a bronchial challenge.

MATERIAL & METHODS

Study group

Eighteen patients, seven women (six in the AIA group) and eleven men, with a diagnosis of asthma and nasal polyposis participated in the study (Table 1). The age was 30-61 years, and the polyp size was graded 1-3 [16]. All patients were selected from the Deptartment of Pulmonary and Allergic Diseases and/or the ENT department of the Karolinska University Hospital of Huddinge. Eleven of the patients had a history suggesting NSAID-intolerant asthma, five had no such history and in two the history regarding NSAID intolerance was unclear.

Table 1. Patient data.

Subject	Age	Sex	AIA/ATA	History AI	FEV_1, % of expected	Asthma duration, years	Cumdose, μmol	PD20, μmol
1	55	F	AIA	yes	87%	25	100	74.9
2	52	F	AIA	yes	87%	13	30	20.8
3	42	M	AIA	yes	91%	4	30	22,7
4	37	M	AIA	yes	82%	16	30	16.5
5	30	M	AIA	yes	94%	15	3	1.6
6	61	F	AIA	yes	64%	37	180	150
7	33	M	AIA	yes	76%	13	10	7.9
8	59	M	AIA	no	85%	–	30	22.1
9	42	F	AIA	yes	91%	15	100	88.7
10	56	F	AIA	yes	83%	20	30	28.7
11	40	F	AIA (oral challenge)	yes	112%	16	–	–
12	43	M	ATA	no	108%	30	–	–
13	60	M	ATA	yes	87%	20	–	–
14	49	M	ATA	no	99%	25	–	–
15	46	M	ATA	no	112%	6	–	–
16	54	M	ATA	no	78%	35	–	–
17	44	M	ATA	no	81%	3	–	–
18	40	F	ATA	no	98%	35	–	–

All but one (AIA-patient) were non-smokers, although four were ex-smokers having stopped at least six years ago. Nine of the 18 patients were allergic, and for this reason two of these had previously been treated with specific immunotherapy. Nine patients had previously had polyp surgery. Their mean age/asthma duration was 46/17 years in the AIA group and 48/22 years, respectively, in the ATA (aspirin-tolerant asthmatic) group.

Before the tests, the subjects had had no airway infections for at least 30 days. All of them gave their informed consent. The local ethics committee approved the study (151:98).

Study design

The patients underwent a bronchial as well as a nasal challenge test with lysine-aspirin, and these tests were performed with a gap of at least 18 days. During tests, the nasal as well as the bronchial responses was continuously evaluated

Before the nasal and bronchial challenge tests, the patients were asked to refrain from oral corticosteroids for 30 days, and leukotriene inhibitors as well as local nasal steroids for at least seven days before the challenge. No β2-agonists or inhaled steroids were allowed for 12 hours before challenge.

Lung function measurements

FEV_1 was measured in a standing position with a Spirolab spirometer (Medical International Research, Rome, Italy). According to the statement of ATS [17], the best value from at least three exhalations was used establishing the baseline value. During the challenge, FEV_1 was recorded every 10 minutes, and the better of two efforts was registered.

Nasal measurements

The RSM-LDF apparatus

The Rhinostereometer with a laser Doppler flowmeter attached (RSM-LDF apparatus, Figure 1) records changes in nasal mucosal swelling and microcirculation simultaneously in a localized area on the mucosa on the inferior turbinate. The Rhinostereometer is an optical apparatus that can determine changes in mucosal swelling in the human nose [18]. To maintain the distance between the area to be studied during the test and the microscope, the patient bites down on an individually-cast tooth splint fixed to the frame, which is attached to the RSM-LDF apparatus, and prevents head movement during the measurements. A tooth splint can easily be made by the investigator. A hard plastic material, commonly used by dentists, is heated in hot water to make it soft, and then fixed on a metal device. The patient to be examined bites carefully down before the plastic material cools down, then it is cooled in water for a short time to harden again after which it is ready for use [14,15,18,19]. The laser Doppler flowmeter is equipped with a specially designed probe attached to a micromanipulator, which allows for a continuous adjustment of the distance to the mucosa, and is kept within 0.3 mm [14,15,19]. The laser Doppler flowmetry apparatus measures the microcirculation in the

Figure 1. The rhinostereometer.

superficial part of the nasal mucosa. Light with a wavelength of 780 nm is transmitted on to the tissue via a fibre optic probe. When the light strikes the moving blood cells, it undergoes a change in wavelength (Doppler shift), which is received by the specific fibres. A computer analyzes the data. The magnitude and frequency distribution of these changes are directly related to the number (CMBC) and mean velocity of moving blood cells in the volume measured, i.e., the blood perfusion. Consequently, VELOCITY x CMBC = PERFUSION. The results are given in arbitrary units, and therefore, the perfusion is expressed in arbitrary perfusion units (PU). PU cannot be given in ml/min/100g tissue, although there is a linear relationship between PU and ml/min/100g tissue [20]. Earlier studies have shown that in the human skin the measurement depths have been estimated to 0.5-1 mm [21], and in the nasal mucosa to about 1 mm [22].

Using the RSM-LDF it is important to obtain stable measurements to achieve a correct baseline value, and therefore the subject was acclimatized to the examination room for at least 30 minutes before the measurements of the baseline values were taken. When the position of the nasal mucosa differed by no more than 0.2 mm in 3 different measurements separated by one minute, the final recordings of the swelling and the microcirculation on each side became the baseline values.

Nasal air flow (PNIF)

PNIF measurements were used only throughout the nasal challenge test. An In-check™ Portable Inspiratory Flow-meter (Clement Clark, Harlow, England) was used to measure the nasal inspiratory airflow. Firstly, the patient was instructed how to use the equipment and when the investigator judged the technique to be satisfactory, the best value from at least three inhalations was used establishing the baseline value. During the challenge, PNIF was recorded every 10 minutes, and the better value of two efforts was registered.

*Nasal symptom score*s

Nasal symptom scores measurements were used only throughout the nasal challenge test. The patients estimated the baseline symptoms, using a visual analogue score by giving a score using a number between 0 and 10. The symptoms were: stuffiness- from free (0) to totally obstructed nose (10) and rhinorrhoea- from dry nose (0) to an intolerably runny nose (10). As the challenge test started, they continued to estimate the symptoms in the same manner. After finishing the test, the change in symptoms in relation to the baseline was calculated.

Lysine-aspirin

Water-soluble aspirin – lysine-aspirin – was used for both the nasal and bronchial challenge.

A lysine-aspirin solution was freshly made immediately before each challenge using Aspisol® (Horby Bayer AG, Leverkussen, Germany). The crystalline powder was dissolved in 0.9% saline to a 1M solution (= 180 mg/mL).

For the bronchial challenge and for the 18 mg nasal provocation, it was then further diluted to a 0.1M solution. For the 36 and 25 mg provocation, it was diluted instead to a 0.2 M solution.

Bronchial challenge

Before starting the bronchial provocation test, the baseline RSM-LDF (rhinostereometer with a laser Doppler flowmeter attached) measurements were performed as described above, and these measurements were repeated 15 minutes after saline inhalation, and 15 and 30 minutes after each lysine-aspirin dose increment. In the AIA group, measurements were continued every 15 minutes until a 20% drop in pulmonary function (PD20), and then at least 60 minutes, and often more than two hours, until the lung function was judged to be recovered. In the ATA group the patients were challenged in the same way, until they completed the entire test, 260 minutes from start.

The bronchial challenge was performed according to the method described by Dahlén et al. [8], using a dosimeter-controlled jet-nebulizer (Spira Elektro 2, Respiratory Care Centre, Haemenlinna, Finland). Powered by compressed air at 7.5 L/min, it generates an aerosol with a mass median diameter of 4.1 μm and with a nebulisation period of 0.8 s, 10.3 μL solution was delivered every breath.

The bronchial provocation started by inhaling nine breaths of NaCl, with measurements of FEV_1 10 and 20 minutes after inhalation. Then, starting 20 minutes after the NaCl inhalation, lysine-aspirin was inhaled creating increasing cumulative doses every 30 minutes. At 10, 20 and 30 minutes after each dose, first the measurements of nasal swelling and microcirculation (RSM-LDF) and then spirometry measurements were performed afterwards, as described above. The challenge was stopped when FEV_1 had decreased by 20% or more compared to FEV_1 20 minutes post-diluent, or when the maximum dose (300 μmol, and the cumulative dose 600 μmol) was reached 260 minutes from start. The broncho-constriction was immediately reversed by inhaling 5 mg Salbutamol (Ventolin®, GlaxoSmithKline, Middlesex, England) and 0.5 mg

Ipratropiumbromid (Atrovent®, Boehringer Ingelheim, Ingelheim, Germany).

Nasal challenge

Lysine-aspirin was applied to the nasal mucosa using a 10 ml spray-pump (pump: Valois, France, bottle: Saint Gobain, France) with a 100 μl volume for each spray. The patients had a single nasal challenge procedure with the same volume instilled/administered bilaterally. To avoid inhalation of lysine-aspirin into the bronchi, the patient was instructed not to breathe through the nose during the spray procedure, and not to lean the head backwards until it was over. The nasal challenge started unilaterally with two sprayings on the lateral wall and immediately thereafter, another two sprayings on the medial wall during apnoea of both cavities. Then, the patients bent their head forward, instructed to refrain from any more nasal breathing to avoid bronchial inhalation. The procedure was repeated in this way on the other side, and until there was no more lysine-aspirin left to spray. The patients in the AIA group were challenged with different doses, 18, 25 or 36 mg lysine-aspirin, while the patients in the ATA group were challenged with only 36 mg. In total, the volume of the lysine-aspirin was enough for about 10-16 sprayings on the nasal mucosa when spraying 18 mg (0.1 M) and 36 mg (0.2 M) and 7-12 sprayings when spraying 25 mg (0.2 M).

After concluding the spray procedure the measurements were repeated every 10 minutes for 180 minutes until the end of test, in the following order: a) estimation of the nasal symptom scores, b) bilateral measurements of the nasal mucosal swelling and the microcirculation, c) PNIF- measurements, d) spirometry with measurements of FEV_1.

Statistics

For comparisons of swelling, microcirculation, PNIF and FEV_1 within and between groups, Mixed Effect Models (SAS® 9.1, Procedure Mixed) were used. For calculating symptom scores, the Wilcoxon-Mann-Whitney test was used for detecting changes between groups. The histamine $PD_{20}FEV_1$ values were calculated from the log-dose response curves by linear interpolation [23].

RESULTS

Bronchial challenge

All 18 subjects completed the bronchial challenge test.

Lung function

Ten subjects had a positive challenge test with a geometric mean PD20 value of 43.4 μmol lysine-aspirin (Table 1). With one subject, the bronchial provocation test was negative, but due to the history, the patient later also had an oral aspirin provocation test, which was judged to be positive. Therefore, 11 subjects were included in the AIA group and seven in the ATA group. Mean baseline FEV_1 in the AIA group was 84% (1.71 – 4.14 L) of expected, and 97% (3.13 – 3.8 L) in the ATA group. Mean FEV_1 20 minutes post-diluent in the ATA group was 3.38 L (2.61-4.43 L), and at the end of the entire provocation test, 260 minutes from start, it had decreased by 3.8% to 3.25 L (2.58-4.04).

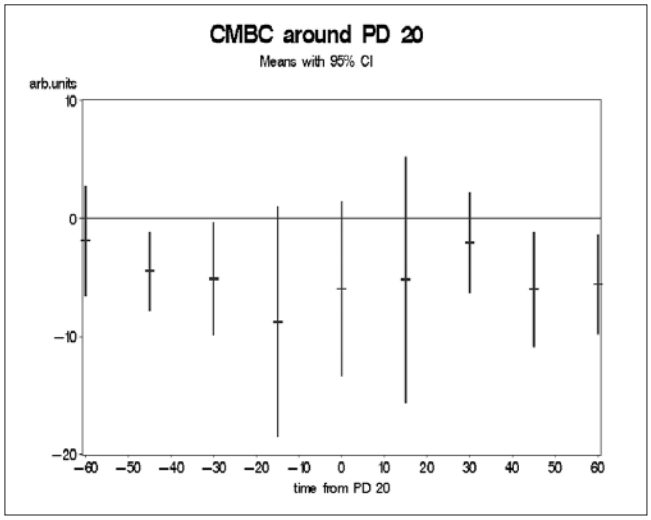

Figure 2. CMBC (concentration of moving blood cells) in the AIA group throughout bronchial challenge test. The nine nasal measurements of CMBC, within the time span 60 minutes before until 60 minutes after PD20 was reached, were decreased (mean 88, range 61-127 arb. units) compared to baseline CMBC (mean 86, range 74-95 arb. units, p=0.041, Mixed Effect Models). 0 in the X- axis illustrates the time when FEV_1 decreased 20 % or more from baseline (mean CMBC 86 arb. units, range 74-95), and the challenge test was stopped. The Y-axis shows the Δ-values of CMBC in relation to baseline.

Nasal measurements

After having performed the bronchial challenge test, one ATA patient did not want any further participation in the study with the nasal challenge test, due to discomfort with the tooth splint, and in one patient in each group the nasal measurements throughout the bronchial challenge test failed, due to technical problems with the equipment.

In the AIA group CMBC (the concentration of moving blood cells) was significantly reduced ($p = 0.041$, Mixed Effect Models) as compared to baseline (mean 86, range 74-95 arb. units) in the interval between 60 minutes before to 60 minutes after the 20% fall in FEV_1 (PD20) and end of the challenge procedure (mean 88, range 61-127 arb. units) (Figure 2). There were no corresponding changes in the measurements of perfusion or swelling ($p > 0.05$). In the ATA group there were no significant changes in the nasal parameters throughout the nasal challenge test.

Nasal challenge

Eleven AIA patients and six ATA-patients participated in the nasal challenge test. When the first seven AIA patients had completed the nasal challenge test with 18 mg lysine-aspirin, only three had increased nasal symptoms and nasal mucosal swelling. The remaining four were therefore re-challenged with

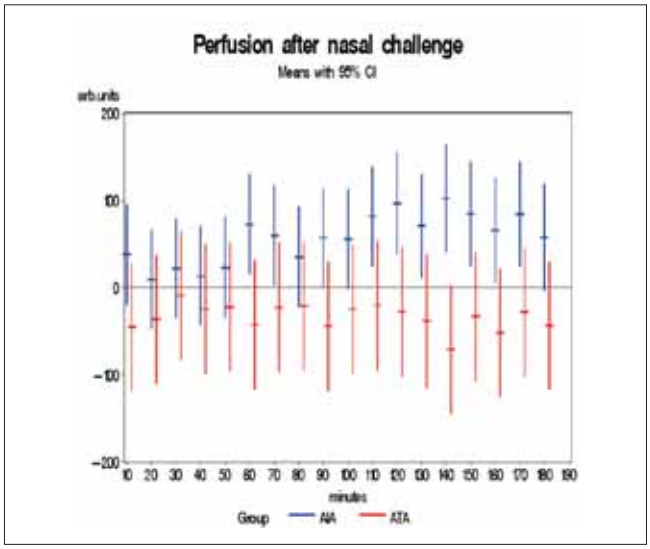

Figure 3. The perfusion in both groups throughout nasal challenge test. The perfusion was significantly increased within the AIA group as compared to the ATA group in the interval 90-180 minutes after nasal challenge (p=0.007, Mixed Effect Models). The Y-axis shows the Δ-values of perfusion in relation to baseline.

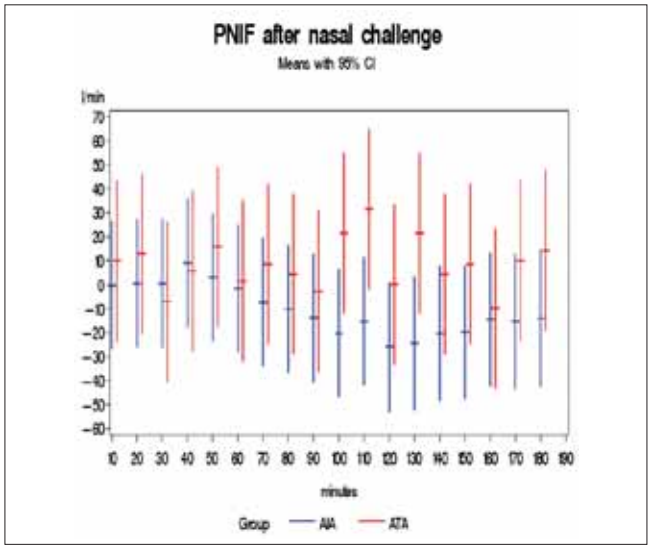

Figure 4. PNIF in both groups throughout nasal challenge test. Mean PNIF was reduced in the AIA group as compared to the ATA group in the interval 100-130 minutes after spray ($p = 0.039$, Mixed Effect Models). The Y-axis shows the Δ- values in relation to baseline.

36 mg lysine-aspirin, as well as the remaining AIA-patients, except for one where the dose was reduced to 25 mg due to low lung function. Consequently, in the AIA group three patients were challenged with 18 mg only, four patients with both 18 and 36 mg, three patients with 36 mg only, and one patient with 25 mg. In contrast, 36 mg was used as the challenge dose for all ATA-patients.

Bronchial measurements

One patient in the ATA group did not perform spirometry throughout the nasal challenge test.

The mean baseline FEV_1 in the AIA group before the nasal challenge test was 87% of that expected (1.58 – 4.11 L), and in the ATA group it was 95% of that expected (2.64 – 4.24 L). However, there were no significant differences between the two groups in FEV_1 at baseline ($p > 0.05$). One patient in the AIA group developed asthma 110 minutes after the nasal challenge (36 mg) and was therefore treated with inhalations of Salbutamol and Ipratropiumbromid.

Nasal measurements

Two patients in the AIA group were studied only 120 minutes after the spray due to logistical reasons; the remaining 15 subjects were studied 180 minutes.

The perfusion was significantly increased in the AIA group (mean 332, range 157-758 arb. units) compared to the ATA group (mean 235, range 45-445) in the interval 90-180 minutes after nasal challenge ($p = 0.007$, Mixed Effect Models, Figure 3).

The nasal swelling was significantly increased ($p < 0.05$, Mixed Effect Models) compared to baseline within the AIA group in the interval 50-180 minutes after nasal challenge (mean 0.6, range -0.19 - 0.29 mm). Although there were no corresponding increases ($p > 0.05$, Mixed Effect Models) in the ATA group (mean 0.2, range -0.11-0.14 mm), there were no significant differences between the two groups ($p = 0.24$).

PNIF was reduced in the AIA group (mean 123, range 50-190 units) as compared to the ATA group (mean 203, range 110-260 units) in the interval 100-130 minutes after spray ($p = 0.039$, Mixed Effect Models, Figure 4).

The symptom scores of patency and rhinorrhoea increased significantly ($p = 0.05$ and $p = 0.025$, Wilcoxon-Mann-Whitney one-sided test, mean 5.2, range 0-10 respectively), and the mean number of sneezes were significantly higher ($p = 0.018$, Wilcoxon-Mann-Whitney one-sided test, mean 2.5, range 1-6) in the AIA group as compared to the ATA group in the time span 90-180 minutes after the sprayings (Figures 5a, b and c).

DISCUSSION

In this study we have prolonged the detection time after nasal challenge with lysine-aspirin from the recommended two hours [3] to three hours, and investigated the effects of challenge on the nasal mucosal blood-flow in a group of aspirin-intolerant asthmatics (AIA).

Firstly, prolonging the detection time from two to three hours revealed that the main reaction occurred about 90-180 minutes after challenge. This is new data because to our knowledge no other study has reported a detection time longer than 120 minutes after nasal challenge [10-12,24,25], and in some studies it has even been shorter [26]. We do not find this late nasal response to lysine-aspirin surprising, because the reaction in aspirin intolerance is complex, and may require more time to develop than for instance the nasal response to histamine challenge,

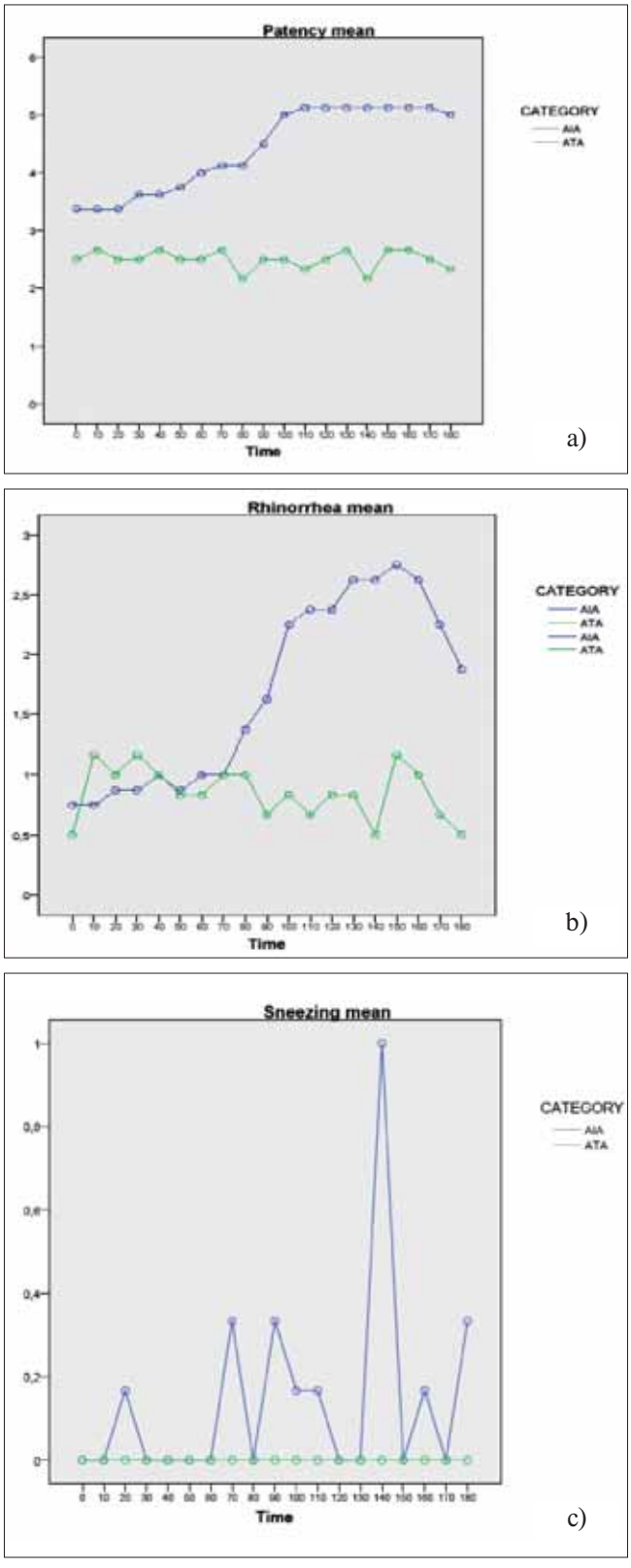

Figure 5. The mean symptom scores of a) patency, b) rhinorrhoea and c) sneezes increased significantly in the AIA group as compared to the ATA group in the time span 90-180 minutes after spray (p= 0.05, p= 0.025, and p= 0.036 respectively, Wilcoxon-Mann-Whitney one-sided test). In Figures 5a and 5b, the Y-axis represents mean score (0-10), and in Figure 5c the Y-axis represents the mean number of sneezes in the last 10 minutes.

where the reaction occurs in connection to the provocation due to the direct effects of histamine on the blood vessels in the nasal mucosa. This data is particularly interesting because the GA2LEN guidelines recommend a measurement period of two hours, unless the patient develops clinical symptoms by the end of two hours of observation in which case a three-hour measurement period is then recommended [3]. Therefore, the outcome of the PNIF-, and RSM-LDF measurements as well as the symptom scores raises the question whether the GA2LEN recommendations of two hours observation time after application of lysine-aspirin might be adjusted, at least for these different assessment methods. Concerning Acoustic rhinometry and Rhinomanometry, the outcome of this study demands further investigation into whether a three-hour detection time might improve the sensitivity of these methods as well. With regard to the dose recommended by GA2LEN, 16 mg, we used higher doses in this study, because it was designed before the GA2LEN recommendations were published [3]. The fact that one of the AIA patients who received both an 18 and a 36 mg dose developed asthma symptoms after being challenged with the higher dose, implies the lysine-aspirin dose according to the GA2LEN Guidelines reduces the risk of a severe asthma reaction.

Secondly, we found a clear-cut reaction in the microcirculation (an increase in the perfusion) after nasal challenge in a group of AIA, and this reaction differed significantly from that of a group of aspirin-tolerant asthmatics (ATA). Consequently, nasal challenge with lysine-aspirin with detections of alterations in blood flow as measured by RSM-LDF might become useful as a complementary diagnostic method for detecting aspirin intolerance. We have previously found that the perfusion increased throughout histamine challenge test as measured by RSM-LDF [27], and it was further increased under inflammatory conditions when a group of healthy subjects had been exposed to swine dust [15]. Consequently, the increased perfusion in the AIA group after nasal challenge could be interpreted as a sign of increased inflammation of the nasal mucosa. The perfusion of flow is the product of the measured, independent parameters concentration (CMBC) and velocity of moving blood cells: PERFUSION = CMBC x VELOCITY. Previously, it has been demonstrated that CMBC decreased after an injection of saline 1 mm into the nasal mucosa [22], and in addition we also found a significant correlation between a decrease in CMBC and increased levels of nasal lavage albumin after swine dust exposure [15]. Our hypothesis therefore is that plasma extravasation can be detected by a decrease in CMBC; in this study occurring at the time the patients developed airway obstruction during the bronchial challenge test. If this data is reproducible, it would be further confirmed by the detection of increased levels of albumin, or the presence of α-2 macroglobulin in nasal lavage after bronchial lysine-aspirin challenge of AIA patients. However, to our knowledge there are no publications of such findings, and therefore it would be

appropriate to perform a study with an assessment of CMBC and plasma proteins in nasal lavage after nasal lysine-aspirin challenge test. The mechanisms behind this possible broncho-nasal connection is unclear, it may be due to a systemic distribution of inflammatory mediators or to a broncho-nasal reflex, which unlike the naso-bronchial reflex is not well established. To our knowledge only two papers, which describe the phenomenon [28,29] have been published.

The advantages of nasal lysine-aspririn challenge are already mentioned in this manuscript; however, the method also has some disadvantages. Massive obstruction of polyps might prevent the lysine-aspirine to reach the nasal mucosa and therefore increase the risk of a false negative outcome. In addition, each detection method puts its limit for the severity of the polyposis. RSM-LDF demands that the investigator can bilaterally visualize the anteriomedial part of the Inferior Turbinate, when using PNIF it is necessary to use certain airflow in the nasal cavity before challenge, and symptoms scores are useless as a detection method if they are scored to the maximum before the challenge. When including the patients, we used anterior rhinoscopy and nasal endoscopy to confirm the diagnosis of nasal polyposis [30], and to make sure that it was technically possible to perform the nasal challenge as well as the nasal measurements. We used PNIF and symptom scores, which are recommended methods in the GA2LEN guidelines, but added RSM-LDF with the purpose of evaluating possible changes in mucosal swelling and microcirculation. The rationale was that acoustic rhinometry, as well as active anterior rhinometry could be described as extensions of the rather blunt measurements of PNIF. One advantage of combining RSM-LDF and PNIF is that RSM-LDF measures a single spot in the nasal mucosa while PNIF evaluates the entire nasal cavity, and in this way these two methods together with detection of symptom scores complement each other. Rhinostereometry is a more exact method of measuring nasal mucosal swelling than acoustic rhinometry and rhinomanometry, and measures changes with a detection limit of 0.1 mm. One drawback with the method is that it requires the patient to be in a calm environment for the nasal mucosa to be as stable as possible, and it also requires multiple measurements for establishing baseline values [14,31]. In addition, it is slightly trickier to use compared with PNIF and even acoustic rhinometry, and therefore it requires some practice before starting the assessments (Figure 1).

Using rhinostereometry, there were no significant differences in nasal mucosal swelling between the groups in contrast to the microcirculation (perfusion), PNIF and the symptom scores. There was, however, a significant increase in swelling as compared to baseline within the AIA group. This is because the onset of the nasal mucosal swelling increase occurred quite early in the test in both groups, and therefore nasal hyperresponsiveness might have caused this reaction. Rhinostereometry has certainly been proven to be a sensitive method for detecting hyperresponsiveness in previous studies of nasal mucosal swelling throughout histamine challenge tests [14,15,32-34]. According to the GA2LEN recommendations [3] nasal saline challenge should be used prior to the lysine-aspirin challenge to discount nasal hyperresponsiveness from the actual lysine-aspirin response. This was not done in our study, because it was performed prior to the GA2LEN recommendations. However, the fact that the main reaction for the perfusion (Figure 3), PNIF (Figure 4) and symptom scores (Figure 5) occurred late in the AIA group suggests that nasal hyperresponsiveness was not a crucial factor for the outcome of these parameters.

ACKNOWLEDGEMENTS

The authors wish to thank Ann-Sofie Lantz and Agneta Gülich for their skilful participation in this study.

The study was sponsored by grants from the Swedish Association of Otorhinolaryngology, Head and Neck Surgery, the Acta Otolaryngologica Foundation, Praktikertjänst AB, Swedish Heart Lung Foundation, Association Against Asthma and Allergy, Medical Research Council (74X-15067) and the Swedish Council for Worklife Research (94-1383).

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