



Acetylsalicylic acid-triggered 15-HETE generation by peripheral leukocytes for identifying ASA sensitivity

Peter Korosec^{a,*}, Ursa Tisler^b, Nissera Bajrovic^a, Mira Silar^a, Ales Mrhar^b, Mitja Kosnik^a

^a University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

^b University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia

KEYWORDS

Acetylsalicylic acid
hypersensitivity;
15-HETE;
Leukocytes

Summary

Background: Exposure to acetylsalicylic acid (ASA) may exacerbate respiratory or skin diseases or induce anaphylactoid reactions in apparently healthy individuals. We wanted to evaluate the clinical and diagnostic utility of measuring ASA-induced 15-hydroxyeicosatetraenoic acid (15-HETE) generation.

Methods: We performed a prospective single-blind study with 26 subjects undergoing clinical evaluation and/or ASA provocation testing. We also included 12 control subjects. Peripheral blood leukocytes were incubated with 500 μ M ASA and 15-HETE release was measured by competitive ELISA.

Results: We found that 18 subjects were ASA-tolerant and 8 were ASA-intolerant. The mean increase in 15-HETE in intolerant subjects was 34% and this was comparable to the mean increase of 30% observed in ASA-tolerant subjects. A similar mean increase was also observed in control subjects. The ROC calculation showed that the optimal diagnostic threshold would be an increase of greater than 33%. However, the sensitivity of this increase was only 63% and the specificity was 50%.

Conclusions: Our data suggest that further studies are needed before the ASA-induced 15-HETE test can be used in clinical practice.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

Exposure to acetylsalicylic acid (ASA) and nonsteroidal anti-inflammatory drugs (NSAIDs) may exacerbate nasal and/or respiratory symptoms in sensitive individuals with asthma, or cutaneous symptoms in patients with chronic urticaria.¹ Urticaria and angioedema may also occur in apparently healthy individuals.² At present, the mechanism of intolerance is not fully understood. The diagnosis of ASA intolerance can be confirmed by patient history or with a provocation test. The test usually takes 2 days and may be associated with severe adverse reactions; thus, alternative procedures with a higher safety profile are highly desirable. Therefore, *in vitro* methods are being developed. One such method is the 15-hydroxyeicosatetraenoic acid (15-HETE)

test. This test is based on previous reports suggesting that the peripheral blood leukocytes from ASA-sensitive asthma patients may be triggered *in vitro* with ASA to generate increased amounts of (15-HETE).^{3–5} For this reason, we conducted a prospective single-blind study on a limited number of patients to evaluate the diagnostic sensitivity and specificity of 15-HETE testing in a routine clinical setting.

Material and methods

We included 26 subjects (mean age 52 years; 18 women, 8 men) recruited for the assessment of hypersensitivity to non-steroidal anti-inflammatory drugs. In 58% of subjects, a placebo-controlled oral provocation test with up to 933 mg of ASA was performed, as previously described.² We also included 12 matched control subjects (mean age 51 years; seven women, five men). A 15-HETE test was carried out and analyzed as previously described.⁴ Briefly, peripheral blood leukocytes were incubated with 500 μ M aspirin (Aspegic,

*Corresponding author. Peter Korosec. University Clinic of Respiratory and Allergic Diseases Golnik, Golnik 36, 4204 Golnik, Slovenia.
E-mail: peter.korosec@klinika-golnik.si (P. Korosec).

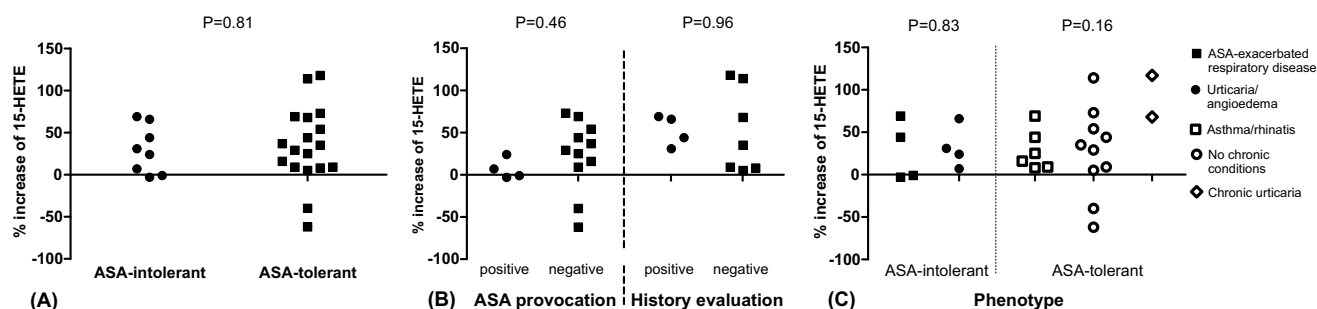


Fig. 1. Effect of ASA on increased generation of 15-HETE production in (A) ASA-tolerant and ASA-intolerant subjects, (B) subjects divided according to their provocation test results and history evaluations, (C) subjects divided according to their clinical phenotypes.

Sanofi-Synthelabo, Quetigny, France) and 15-HETE release was measured in cell supernatants with competitive ELISA (Assay Designs Inc., Ann Arbor, MI, USA).

Results

We found that 18 subjects were ASA-tolerant and 8 subjects were ASA-intolerant. Diagnosis was confirmed by an ASA provocation test in 11 out of 18 tolerant patients and four out of eight intolerant ones. In others, the diagnosis was based on a strongly positive history of ASA intolerance, such as a clinical reaction clearly documented by a physician or the notation of more than one event. In the ASA-intolerant group, four subjects had asthma and/or rhinitis; in these subjects the sensitivity was manifested as exacerbation of nasal and/or respiratory symptoms; in the other four subjects, the sensitivity manifested as urticaria-angioedema, although none of them had previous chronic respiratory or skin disease. In the ASA-tolerant group, six subjects had asthma and/or rhinitis and two had chronic urticaria. Peripheral blood leukocytes from ASA-tolerant, ASA-intolerant, and control subjects generated similar amounts of 15-HETE at baseline (7.69 ± 1.36 , 8.22 ± 1.79 , and 10.5 ± 1.43 pg/mL, respectively; $P = 0.37$). Preincubation with ASA increased the generation of 15-HETE to 9.12 ± 1.31 , 9.64 ± 1.79 , and 12.5 ± 1.41 pg/mL, respectively ($P = 0.87$). In ASA-tolerant subjects the increase was 34%, which was comparable to the 30% increase observed in ASA-intolerant subjects (Fig. 1A, $P = 0.81$). Similarly, the mean increase in control subjects was 27% ($P = 0.87$). No difference was observed whether the subjects were divided according to the ASA provocation results, anamnestic evaluation (Fig. 1B, $P \geq 0.46$), or analyzed according to different phenotypes. Namely, both ASA-exacerbated respiratory disease ($N = 4$) and the urticaria-angioedema subgroup ($N = 4$) showed comparably low results (Fig. 1C, $P \geq 0.16$). To establish the sensitivity and specificity of the ASA-triggered 15-HETE release, receiver operating curve (ROC) analysis was also performed. The ROC curve area was 0.57 and the P value was 0.58. The optimal recalculated threshold for ASA-induced 15-HETE increase was $> 33\%$, with a true positive rate of 63% (sensitivity) and a false positive rate of 50% (specificity).

Discussion

Several in vitro assays have been proposed to confirm ASA sensitivity. Those methods are the basophil CD63 activation test,⁵⁻⁷ the ASA triggered release of Cys-LTs

from peripheral blood leukocytes,^{8,9} and the 15 HETE-test.^{3,4,10} Previous publications give conflicting results on the basophil activation test, with some authors showing that ASA basophil testing is diagnostically useful,⁵ whereas others show that it is not useful⁶ or that it is useful only in a subgroup of subjects that experience anaphylactoid reactions.⁶ Second, although the leukocytes of patients with aspirin sensitivity produce higher amounts of Cys-LTs as measured by CAST, the differences between ASA-intolerant and ASA-tolerant subjects were merely quantitative and the test's sensitivity to detect ASA intolerance rarely exceeded 50%.^{8,9} Furthermore, because the predictive value of CAST was also low,⁹ the test has limited clinical usefulness in the diagnosis of ASA hypersensitivity.

15-HETE is a potential inflammatory mediator, although the mechanisms leading to increased generation of 15-HETE after in vitro incubation with ASA are not known. In addition to 15-lipoxygenase, both COX-1 and COX-2 are capable of synthesizing 15-HETE, and ASA treatment stimulates the formation of 15-HETE by COX-2 (but not COX-1), although it inhibits prostaglandin generation.³ The evidence in favor of the use of 15-HETE testing is two reports from 2000¹⁰ and 2003³ that documented specific triggering of 15-HETE generation by ASA in peripheral blood and nasal polyp epithelial cells from intolerant asthma patients. The same group then assessed the possible diagnostic value of ASA-induced 15-HETE generation. In their third report from 2005,⁴ they suggested that the sensitivity and specificity of 15-HETE testing to detect ASA intolerance in asthma and rhinitis patients was high, reaching 80%. Unfortunately, these results have not been reproduced by other research groups to date.

In conclusion, we showed comparable and moderate increases in 15-HETE after in vitro incubation with ASA in ASA-tolerant and ASA-intolerant subjects and also in control subjects. Therefore, we demonstrated limited usefulness of ASA 15-HETE testing for the diagnosis of ASA sensitivity. Further evaluation of the test in a larger number of well-characterized patients is needed before the 15-HETE test can be used in clinical practice.

Conflict of interest statement

The authors declare that they have no competing interest.

References

1. Kowalski LM, Makowska SJ. Aspirin-exacerbated respiratory disease - an update on diagnosis and management. *Allergy Clin Immunol Int J World Allergy Org* 2006;18:140-9.

2. Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczynska M, Picado C, Scadding G, et al. EAACI/BA2LEN guideline: aspirin provocation test for diagnosis of aspirin hypersensitivity. *Allergy* 2007;**62**:111-8.
3. Kowalski ML, Ptasińska A, Bienkiewicz B, Pawliczak R, DuBuske L. Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic-acid generation by leukocytes from aspirin-sensitive asthmatic patients. *J Allergy Clin Immunol* 2003;**112**:505-12.
4. Kowalski ML, Ptasińska A, Jedrzejczak M, Bienkiewicz B, Cieslak M, Grzegorzczak J, et al. Aspirin-triggered 15-HETE generation in peripheral blood leukocytes is a specific and sensitive Aspirin-Sensitive Patients Identification Test (ASPI-Test). *Allergy* 2005;**60**:1139-45.
5. De Weck AL, Sanz ML, Gamboa PM, Aberer W, Blanca M, Correia S, et al. Nonsteroidal anti-inflammatory drug hypersensitivity syndrome. A multicenter study. I. Clinical findings and in vitro diagnosis. *J Invest Allergol Clin Immunol* 2009;**19**:355-69.
6. Bavbek S, Ikinciogullari A, Dursun AB, Güloğlu D, Arkan M, Elhan AH, et al. Upregulation of CD63 or CD203c alone or in combination is not sensitive in the diagnosis of nonsteroidal anti-inflammatory drug intolerance. *Int Arch Allergy Immunol* 2009;**150**:261-70.
7. Korosec P, Mavsar N, Bajrovic N, Silar M, Mrhar A, Kosnik M. Basophil responsiveness and clinical picture of acetylsalicylic acid intolerance. *M. Int Arch Allergy Immunol* 2011;**155**:257-62.
8. Pierzchalska M, Mastalerz L, Sanak M, Zazula M, Szczeklik A. A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leukocytes precludes its value for aspirin sensitivity testing in asthma. *Clin Exp Allergy* 2000;**30**:1785-91.
9. Bavbek S, Dursun AB, Birben E, Kalayci O, Misirligil Z. Cellular allergen stimulation test with acetylsalicylic acid-lysine is not a useful test to discriminate between asthmatic patients with and without acetylsalicylic acid sensitivity. *Int Arch Allergy Immunol* 2009;**149**:58-64.
10. Kowalski ML, Pawliczak R, Wozniak J, Siuda K, Poniarowska M, Iwaszkiewicz J, et al. Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am J Respir Crit Care Med* 2000;**161**:391-8.