RESEARCH NOTE

A metabolite of prostaglandin D₂, 11β-prostaglandin F₂α (11β-PGF₂α), in exhaled breath condensate and serum of asthmatics with airway hyperresponsiveness to distilled water [version 1; referees: 2 not approved]

Eduard V. Nekrasov, Juliy M. Perelman, Denis E. Naumov, Anna G. Prikhodko, Elena V. Ushakova, Victor P. Kolosov

Far Eastern Scientific Center of Physiology and Pathology of Respiration, Federal Agency for Scientific Organizations, Blagoveshchensk, Russian Federation

Abstract

This study aims at identifying prostaglandin D₂ (PGD₂) involvement in osmotic airway hyperresponsiveness of asthmatics. PGD₂ primary plasma metabolite, 11β-PGF₂α, was analyzed in exhaled breath condensate (EBC) in response to ultrasonically nebulized distilled water (UNDW) and in serum in asthmatics with different airway response to the hypoosmotic stimulus. The total group of asthmatics (n=27) had a lower basal level of 11β-PGF₂α (0.38±0.13 pg/ml, mean±SEM) in EBC compared to a group of healthy subjects (0.86±0.31 pg/ml, n=5), which decreased following the UNDW challenge to 0.30±0.09 and 0.53±0.12, respectively. The group of asthmatics with airway hyperresponsiveness to UNDW (≥10% FEV₁ drop from baseline, n=14) had a lower concentration of the metabolite (0.28±0.14 pg/ml) as compared to the group without hyperresponsiveness (0.49±0.31 pg/ml, n=10). The 11β-PGF₂α concentration decreased in the both groups after the challenge: 0.20±0.04 and 0.23±0.07 pg/ml in the groups with and without hyperresponsiveness to UNDW, respectively. Serum content of 11β-PGF₂α was ranging from 0 to 61 pg/ml in asthmatics (n=17) and from 7.3 to 85.4 pg/ml in healthy subjects (n=8). It was lower in the group with airway hyperresponsiveness to UNDW (8.4±1.7 pg/ml, n=9) than in the group without the hyperresponsiveness (21.0±8.8 pg/ml, n=8). The obtained results do not support the involvement of PGD₂ in the pathophysiology of asthma with airway hyperresponsiveness to a hypoosmotic stimulus unless other conversions of the prostaglandin occur in the airway under these conditions with formation of metabolites different from 11β-PGF₂α.
Corresponding author: Eduard V. Nekrasov (ed_nekrasov@mail.ru)

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Introduction
Prostaglandin D$_2$ (PGD$_2$) is produced by mast cells and macrophages increasing in response to allergen exposure. In asthmatics, PGD$_2$ affects the airways by causing bronchoconstriction, vasodilation, increasing capillary permeability and mucous production. The role of the prostaglandin in asthmatics with osmotic airway hyperresponsiveness is ill-defined. PGD$_2$ is an unstable compound rapidly metabolized with 11β-PGF$_{2α}$ being its primary plasma metabolite. The aim of this study was estimation of 11β-PGF$_{2α}$ in exhaled breath condensate (EBC) in response to ultrasonically nebulized distilled water (UNDW) and serum in asthmatics with different airway response to the hypoosmotic stimulus.

Methods
The study protocol was approved by the Biomedical Ethics Committee of the Far Eastern Scientific Center of Physiology and Pathology of Respiration (permit #91-1 of 12.01.2015). 39 patients with mild to moderate asthma and 13 healthy subjects participated in the study. The patients were recruited from the Center’s in-patient facilities or invited for follow-up checks. EBC was collected from 27 asthmatics and 5 healthy subjects before and after 3 min provocation with UNDW. Aerosol (the particle size range 0.5–10 μm, average particle size 3 μm) was generated by a Thomex L-2 ultrasonic nebulizer (Poland) operated at 4.5 ml/min. The hypoosmolar challenge test consisted of two consecutive inhalations for 3 min each. The first inhalation was with 30 ml of isotonic solution (0.9% NaCl), and the second with the same volume of distilled water. The temperature of the solution was maintained at 37.3°C. During the inhalation, a participant used a nose-clip and was breathing in a calm manner through a mouthpiece connected via a two-way valve to the container with a solution. Lung function testing was conducted with a spirometer Easy on-PC (ndd Medizintechnik AG, Switzerland) before the provocation test (baseline) and at 1 and 5 min of the recovery period. A drop in forced expiratory volume in 1 sec (%ΔFEV1) of 10% or more from baseline after inhalation with UNDW was considered as airway hyperresponsiveness to the hypoosmotic stimulus. Patients who experienced airway responsiveness to isotonic solutions (n=3) were excluded from the test with UNDW, and they were not included in any group except the total group of asthmatics. Patients who experienced any airway responsiveness were given 2 doses of a selective β$_2$-adrenoceptor agonist to prevent bronchoconstriction. EBC was collected during tidal breathing with a condensing device ECoScreen 2 (Erich Jaeger, Germany). Serum was obtained from 17 patients and 8 healthy subjects before the provocation test. The collected samples were stored at -70°C until analysis. The concentration of 11β-PGF$_{2α}$ was measured by 11β-prostaglandin F$_{2α}$ EIA kit (No. 516521, Cayman Chemical Company, USA) in EBC after freeze-drying and in serum after purification by solid phase extraction on C18 cartridges (Strata C18-E, 55 μm, 70A, 500 mg/6 ml, Phenomenex, USA) as recommended by the manufacturer [Cayman’s kit booklet. URL: https://www.caymanchem.com/pdfs/516521.pdf]. The EIA kit utilizes 11β-PGF$_{2α}$-specific rabbit antiserum, and mouse anti-rabbit monoclonal antibody. Each sample was assayed in duplicate. The optical densities of the samples were used to calculate the concentrations of 11β-PGF$_{2α}$ using an automated Excel spreadsheet [A. Swart 2012–2015. URL: http://www.rheumatologie-neuss.net/index_files/RheumatologieNeuss13.htm].

Results

Dataset 1. Data for calibration curves
http://dx.doi.org/10.5256/f1000research.8084.d115417
Standard Bound/Maximum Bound (%B/B$_0$) values were obtained for the concentrations of the standard (11β-PGF$_{2α}$ pg/ml) which were used to build calibration curves for calculations of 11β-PGF$_{2α}$ content in EBC (Table 1) and serum (Table 2) samples. The values were produced with different EIA kits on different days.

Dataset 2. 11β-PGF$_{2α}$ content (pg/ml) in exhaled breath condensate (EBC) of individual subjects before and after provocation with ultrasonically nebulized distilled water and change in forced expiratory volume in 1 sec (%ΔFEV1 from baseline) after the provocation
http://dx.doi.org/10.5256/f1000research.8084.d115418
N.D. – not determined; UNDW - ultrasonically nebulized distilled water.
Note: Patients who had reaction to 0.9% NaCl were excluded from the test with ultrasonically nebulized distilled water.

Dataset 3. 11β-PGF$_{2α}$ content (pg/ml) in serum of individual subjects before provocation with ultrasonically nebulized distilled water and change in forced expiratory volume in 1 sec (%ΔFEV1 from baseline) after the provocation (if applicable)
http://dx.doi.org/10.5256/f1000research.8084.d115419
N.D. – not determined

The level of 11β-PGF$_{2α}$ in EBC was below the announced detection limit (80% B/B$_0$) of the kit (5.5 pg/ml). For the estimation of the 11β-PGF$_{2α}$ level in EBC before and after the provocation test and comparison of the changes in the groups of healthy subjects and asthmatics with and without airway hyperresponsiveness to UNDW, calibration curves were built using the 11β-PGF$_{2α}$ standard in the range of 0–25.6 pg/ml with additional dilutions of the standard down to 0.64 and 0.256 pg/ml and plotting %B/B$_0$ vs. 11β-PGF$_{2α}$ concentration. Obtained values of %B/B$_0$ for the dilutions were different from 100% (Dataset 1, Table 1).

As a result, the calculated content of 11β-PGF$_{2α}$ in EBC was in the range of 0–3.1 pg/ml (Table 1). The total group of asthmatics had a lower basal level of 11β-PGF$_{2α}$ (0.38±0.13 pg/ml, mean±SEM) compared to the group of healthy controls (0.86±0.31 pg/ml), which further decreased following the UNDW challenge to 0.30±0.09 and 0.53±0.12, respectively. The group of asthmatics with airway hyperresponsiveness to UNDW was found to have a lower
The concentration of the metabolite (0.28±0.14 pg/ml) as compared to the group without the hyperresponsiveness (0.49±0.31 pg/ml). The 11β-PGF₂α content decreased in both groups after the challenge: 0.20±0.04 and 0.23±0.07 pg/ml in the groups with and without hyperresponsiveness to UNDW, respectively.

The content of 11β-PGF₂α in serum was higher ranging from 0 to 61 pg/ml in the total group of asthmatics and from 7.3 to 85.4 in healthy subjects (Table 1). Once again, it was lower on average in the total group of asthmatics than in the healthy subjects (14.3±4.3 vs. 33.7±12.1 pg/ml), and lower in the group with airway hyperresponsiveness to UNDW (8.4±1.7 pg/ml) than in the group without hyperresponsiveness (21.0±8.8 pg/ml). Due to the high variation of 11β-PGF₂α content in the subjects, all differences were statistically insignificant (p>0.05).

Since prostaglandin D₂ is considered to be a mast cell- and macrophage-specific eicosanoid, the lack of an increase in the concentration of its major metabolite 11β-PGF₂α found in the present study suggests a diminished role of these immune cells in the pathogenesis of the inflammatory reaction in asthma patients with osmotic airway hyperresponsiveness. However, the formation of different metabolites of PGD₂, apart from 11β-PGF₂α, have been reported which may possess different physiological activities.

### Conclusion

The obtained results do not support the involvement of PGD₂ in the pathophysiology of asthma with airway hyperresponsiveness to a hypoosmotic stimulus unless other conversions of the prostaglandin occur in the airway under these conditions with formation of metabolites different from 11β-PGF₂α. It would be interesting to investigate the level of other possible metabolites of PGD₂.

### Data availability

_F1000Research:_ Dataset 1. Data for calibration curves, 10.5256/f1000research.8084.d115417

_F1000Research:_ Dataset 2. 11β-PGF₂α content (pg/ml) in exhaled breath condensate (EBC) of individual subjects before and after provocation with ultrasonically nebulized distilled water and change in forced expiratory volume in 1 sec (% ΔFEV1 from baseline) after the provocation, 10.5256/f1000research.8084.d115418

_F1000Research:_ Dataset 3. 11β-PGF₂α content (pg/ml) in serum of individual subjects before provocation with ultrasonically nebulized distilled water and change in forced expiratory volume in 1 sec (% ΔFEV1 from baseline) after the provocation (if applicable), 10.5256/f1000research.8084.d115419

### Consent

The study protocol was approved by the Biomedical Ethics Committee of the Far Eastern Scientific Center of Physiology and Pathology of Respiration (permit #91-1 of 12.01.2015), and the participants gave written informed consent.

### Author contributions

AGP – design of the protocol, the recruitment and clinical investigation of the subjects, data collection and analysis, approval of the manuscript. DEN and EVU – method development, data acquisition, approval of the manuscript. EVN – sample processing (lyophilisation, purification by SPE), data analysis, drafting of the manuscript. JMP – leader of the project, conception and design of the study, critical revision of the manuscript, final approval of the version. VPK – conception of the study, final approval of the manuscript.

### Competing interests

No competing interests were disclosed.

### Grant information

The research was supported by Russian Scientific Foundation (grant No.14-25-00019 was awarded to the Far Eastern Scientific Center of Physiology and Pathology of Respiration, the leader of the project is Dr. J.M. Perelman).

_I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript._

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References


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Version 1

Referee Report 09 February 2017

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Giuseppe Santini
Department of Pharmacology, Faculty of Medicine, Catholic University of the Sacred Heart, Rome, Italy

The critical point of this article is the detection limit of the ELISA KIT used. The concentration of metabolite measured in EBC was too much low. I suggest to concentrate the sample by evaporation under vacuum, or use an another KIT with a higher sensitivity.

The number of healthy subjects from which it was collected EBC is too low. I suggest of collect others EBC samples from healthy subjects.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 01 November 2016

doi:10.5256/f1000research.8696.r17321

Andras Bikov
Department of Pulmonology, Semmelweis University, Budapest, Hungary

I read the article with great interest. Lipid metabolites play a role in the pathophysiology of asthma and their changes during different stimuli are of interest.

1. The critical point of the article is that the EBC 11β-PGF\(_{2α}\) levels were below the lower detection limit of the ELISA kit used in the study. Thus, the authors can simply not conclude on the results as they might have experienced changes and differences on variations of zero (distilled water)… To resolve this crucial error, I recommend concentrating the sample (i.e. vacuum evaporation), or adding a known concentration of standard to each sample to overcome the detection limit.

2. The article is too short. Methodology of EBC collection is extremely variable, and to compare results with external findings, EBC methodology needs to be decribed in more detail.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.
Competing Interests: No competing interests were disclosed.