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Inflammation Research

Evaluation of ECP release from intact tissue biopsies from patients with nasal polyps

A. Behnecke[1], S. Mayr[1], B. Schick[1], H. Iro[1] and M. Raithel[2]

[1] Department of Otolaryngology, Head- and Neck Surgery, Friedrich-Alexander University Erlangen-Nuremberg, Waldstr.1, 91054 Erlangen, Germany, Fax: ++49 91318536857, e-mail: Susanne.Mayr@uk-erlangen.de
[2] Functional Tissue Diagnostics, Department of Medicine I, Friedrich-Alexander University Erlangen-Nuremberg, Ulmenweg 18, 91054 Erlangen, Germany

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Introduction

Rhinosinusitis (CRS) is a common illness [1] and 1–2 % of Europeans are affected by CRS associated with nasal polyps [2]. Reasons for developing nasal polyps are still not completely elucidated but are often associated with other diseases such as Aspirin Intolerance Syndrome. Tissue eosinophilia is a characteristic feature in nasal polyps and the degree of eosinophilia has been considered to correlate with the intensity of disease. Eosinophilic cationic protein (ECP) is a mediator released by activated eosinophils and is used as a keystone marker for nasal inflammation. Investigation of functional intact tissue under ex-vivo conditions using mucosa oxygenation (biopsy mucosa oxygenator®; INTESTINO-DIAGNOSTICS, Erlangen) [3] has the ability to detect cell specific mediator release such as ECP. Mucosa oxygenation has been previously established in gastrointestinal disease. This study was to assess the applicability of this system in analysis of mediator release from eosinophils in nasal tissue.

Material and methods

This study had the approval from the local ethics committee and included 30 biopsies from 5 patients with CRS from polyp tissue and lower turbinate, taken during sinus surgery. Spontaneous ECP secretion as well as tissue ECP content was measured from biopsies using biopsy mucosa oxygenator® [3].

After measuring the wet-weight of biopsies, they were separately placed (<10 s after removal) in modified Hanks' solution to measure spontaneous mediator release during 5 h. Incubation medium contained 4 ml of a Hanks' solution with 25 mM Hepes buffer, 1 % FCS and 0.3 % HSA. To avoid ischemic conditions, the solution was bubbled with a steady flow of room air, ensuring sufficient oxygen pressure inside the tissue of 85–90 mmHg, pH 6 at 37 °C [4]. After incubation of 0 min, 30 min, 60 min, 1 h, 2 h and 5 h, 400 µl of supernatant was removed. Aliquots were immediately put on ice, centrifuged (7 min, 4 °C, 400 g) and stored (–20 °C) until measurement of mediators (ImmunoCAP System, Phadia, Freiburg, Germany).

Amounts of measured ECP release were calculated for the actual incubation volume. ECP release is given as net mediator secretion (ng/ml.mg wet-weight (ww)) ±standard error of the mean (SEM). Statistical analysis was made by 2-sided Wilcoxon test, significance was defined as $p < 0.05$.

After 5 h of mucosa oxygenation, each biopsy was mechanically homogenized [5]. This consisted of three steps: (A) 1,500 µl double-distilled water (1,500 rpm, 5 min), (B) 1,500 µl double-distilled water (1,500 rpm, 3 min) and (C) 1,500 µl modified Hanks' medium supplemented with albumin, PIPES, HEPES buffer and FCS. Steps B and C were to wash teflon homogenisation tube (from A), as previous studies had shown mediator binding. Aliquots were separately frozen at –20 °C until ECP measurement. Total tissue ECP content (ng ECP/mg ww, mean ±SEM) from one sample was calculated by addition of mediator amounts detected in A–C in addition to net ECP release.

Results and discussion

The amount of ECP spontaneously released from vital nasal biopsies increased continuously in polyp tissue during 5 h of mucosa oxygenation. There was a significant difference between nasal polyp and lower turbinate. Spontaneous ECP release from normal turbinate tissue was similar in all individuals over all time points (average net release 0.23 ± 0.12). Biopsies from nasal polyps showed increasing rates of ECP release (net release 5.33 ± 3.40 after 5 h) with a significant increase compared to normal turbinate tissue after 10 min of mucosa oxygenation (Fig. 1). Steady ECP release from polyps throughout the incubation period confirms that mucosa oxygenation can maintain vitality of nasal tissue. Like in gastrointestinal tissue [5], mucosa oxygenation may allow functional studies on nasal tissue to detect local stimulants for eosinophil or mast cell degranulation (e.g. allergens, moulds, bacterial superantigens, salicylates, …).

Evaluation of total tissue content of ECP from all biopsies of nasal polyps revealed significantly enhanced levels of protein compared to lower turbinate (Fig. 2). This supports the infiltration of activated eosinophils into sinus mucosa observed in various sinus diseases.

Although sampling may raise initial ECP release by traumatising tissue, this investigation revealed that samples se-

Correspondence to: S. Mayr

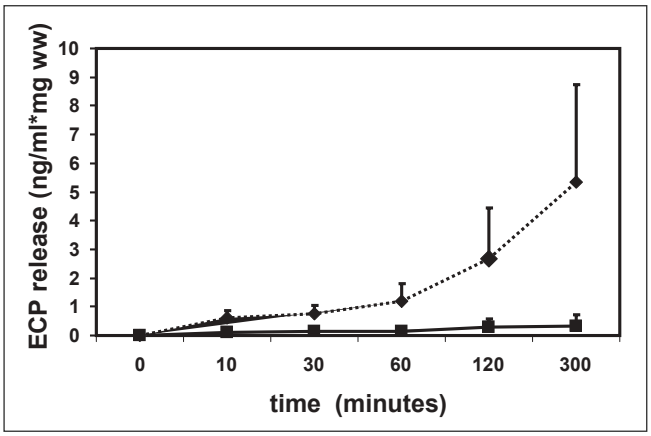

Fig. 1. Spontaneous ECP release from nasal polyp (n = 30, dashed line) and normal turbinate biopsies (control) (n = 30, continuous line) over time (min); $p < 0.05$.

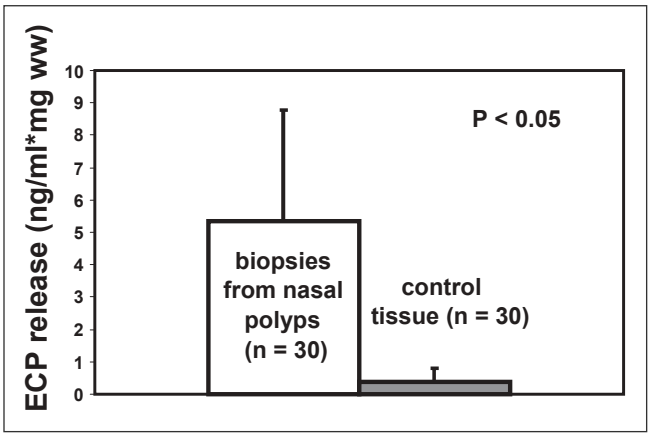

Fig. 2. Total ECP release throughout 5 h; $p < 0.05$.

crete no measurable amounts of ECP directly after sampling. Further work on whether newly formed [5] or granular stored soluble factors are washed out of polyp tissue during mucosa oxygenation and which stimulants are responsible for eosinophil activation [6, 7] will be undertaken.

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