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In vitro effect of glucocorticoids on nasal polyps

Fabiana Valera[1], María S. Brassesco[2], Angel M.Castro-Gamero[3], Maria A. Cortez[4], Rosane G. P. Queiroz[5], Luiz G. Tone[6], Wilma T. Anselmo-Lima[7]

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Abstract

Glucocorticoids are considered the main treatment option for nasal polyps, but their effect is only recently being understood.

Aim: To evaluate whether fluticasone propionate (FP) inhibits the inflammatory process induced by TNF-alpha *in vitro*, and to assess if NF-kappaB is associated to this inhibition.

Study Design: Experimental *in vitro* study.

Materials and Methods: Nasal polyp fibroblasts were cultured during 24 hours. Three different concentrations of FP (1, 10 and 100 nM, added to TNF-alpha) were compared to negative (without additive) and positive (TNF-alpha) controls. Gene expression (RTQ-PCR) and protein concentration (ELISA) of VCAM-1, ICAM-1, eotaxin and RANTES were measured, as well as the nuclear translocation of NF-kappaB.

Results: TNF-alpha significantly increased protein concentration and RNA expression of all the studied molecules, as well as the nuclear translocation of NF-kappaB, when compared to the negative control. FP decreased these parameters in a dose-dependent manner, statistically different from positive control up to 100nM.

Conclusions: FP extensively inhibited inflammatory recruiters, at both protein and RNA levels, confirming the ability of glucocorticoids to modulate the inflammatory process in nasal polyps. This inhibition was associated to decreased NF-kappaB nuclear translocation, demonstrating that this is an important mechanism of glucocorticoids action for nasal polyps.

[1] PhD, MD (professora doutora da FMRP - USP).
[2] PhD (jovem pesquisadora associada ao Laboratório de Pediatria da FMRP-USP).
[3] Geneticist (mestrando no Laboratório de Pediatria da FMRP-USP).
[4] Geneticist (doutora pelo Laboratório de Pediatria da FMRP).
[5] PhD (coordenadora do Laboratório de Pediatria da FMRP).
[6] PhD, MD (professor titular da FMRP).
[7] PhD, MD (professora associada da FMRP).
School of Medicine of Ribeirão Preto - University of São Paulo.
Send correspondence to: Fabiana C. P. Valera - Av. Bandeirantes, 3900 - 12º andar. Ribeirão Preto - SP. CEP 14049-900.
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INTRODUCTION

Nasal polyps (NP) is an inflammatory disease, which primarily affects the sinonasal mucosa[1,2]. Inflammation is generally induced by pro-inflammatory cytokines, such as TNF-α (tumor necrosis factor-α) and IL-1β (interleukin-1β) and mediated by transcriptional factors (TF), such as NF-κβ (nuclear factor-κβ) and AP-1 (activator protein-1)[3]. Initially, TFs translocate into the nucleus and induce the expression of pro-inflammatory molecules in nasal structural cells (fibroblasts, epithelial and endothelial cells)[3-7], which in turn produce chemokines and adhesion molecules (including eotaxin), RANTES (regulated upon activation normally T-expressed and secreted), ICAM-1 (intercellular adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), E-selectin and P-selectin), that will induce the migration of inflammatory cells (such as T lymphocytes, eosinophils, mast cells, and neutrophils) towards the target[7-9]. Once on the nasal mucosa, the circulating cells will promote tissue damage[8,10-12]. Bachert et al.[2] stated that deregulation of chemokines and adhesion molecules production might be important in promoting the local chemotaxis of eosinophils.

According to the European Position Paper on Rhinosinusitis and Nasal Polyps 2007[11] and other researchers[12-15], topical glucocorticoids (GC) are the cornerstone for treating NP. However, the success rate of topical GC ranges from 60.9 to 80%[12,13,16]. The anti-inflammatory property of GCs occurs due to their binding to the glucocorticoid receptor (GR); the GC-GR complex inhibits other TF, such as NF-κβ, a phenomenon known as transrepression[4,17-19]. The repression of NF-κβ will finally inhibit the expression of some cytokines as TNF-α, IL-1, IL-8 (interleukin-8) and ICAM-1[17]. Transrepression between GCs and TFs is reciprocal, and NF-κβ[17] can also repress glucocorticoid receptors; which could, at least in part, induce GC resistance in some patients.

Recently[20], our group has observed that *in-vivo* budesonide treatment leads to a significant improvement in symptoms and endoscopic reduction of nasal polyps. Despite this significant clinical improvement, only one patient reached complete remission. Moreover, we observed that patients with an unfavorable response to clinical treatment presented higher levels of NF-κβ, ICAM-1 and IL-1β expression than before treatment[21]. Since NF-κβ induces transcription of both IL-1β and ICAM-1, we hypothesized that NF-κβ could be considered a pivotal mediator for the initiation of NP and resistance to GC.

Thus, the aim of this study is to observe whether TNF-α induces the expression of inflammatory recruiters in polyp-derived fibroblasts, and whether fluticasone propionate (FP) inhibits this inflammatory response, in an *in vitro* model. Additionally, the mechanism by which this glucocorticoid acts was evaluated through protein and mRNA levels of VCAM-1, ICAM-1, eotaxin and RANTES, as well as by nuclear translocation of NF-κβ.

MATERIALS AND METHODS

The study delineation is summarized in Figure 1. Samples from six patients indicated to surgery after clinical treatment failure were studied. For each case bilateral inflammatory NP was confirmed by CT scans and nasal endoscopy. Patients with associated systemic diseases such as ciliary dyskinesia, cystic fibrosis, AERD (aspirin exacerbated respiratory disease) or severe asthma were excluded. Prior to surgery all patients were kept free of any medication for one month. The present study was approved by the local IRB (process number 4374/2007).

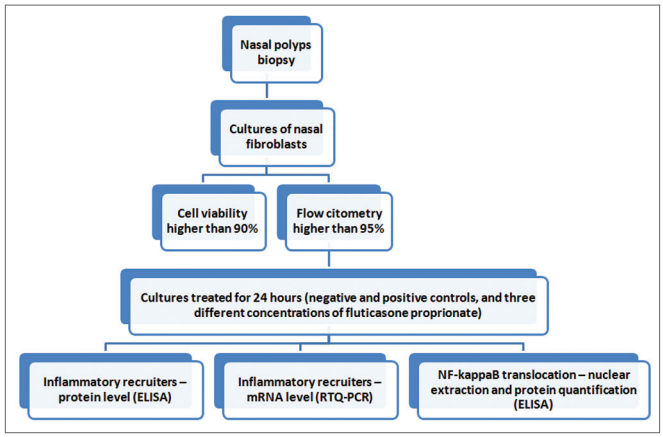

Figure 1. Fluxogram of the study protocol

Cell culture

During the surgical procedure, a polyp biopsy was aseptically collected. The polyps were minced into 0.5 mm fragments with surgical knife, and the fragments were then disaggregated with collagenase type IV for 2 hours. Following the enzymatic treatment, the cells were centrifuged and the collagenase solution removed and replaced with culture medium HAM-F10 (supplemented with 1% penicillin, 1% streptomycin, and 20% fetal calf serum), and cultured at 37°C in a 5% CO_2 atmosphere.

After reaching 90% of confluence (one million cells/flask) the cells were trypsinized and the fibroblast component was confirmed by flow cytometry, following the protocol of Saalbach et al.[22].

Polyp cultures containing at least 90% fibroblasts were then replicated into 5 different flasks and after 24hs treated as follows: negative control (without additive), positive control (TNF-α 25ng/mL), FP1 (TNF-α 25ng/mL and FP 1nM/ 0.45μg/mL), FP10 (TNF-α 25ng/mL and FP 10nM/4.5μg/mL) and FP100 (TNF-α 25 ng/mL and FP at 100nM/45μg/mL). These flasks, as their duplications, were incubated at 37°C in a 5% CO_2 moist atmosphere