Unusual Secretion of Eosinophil Mediators Induced by Benralizumab

Running title: Eosinophil secretion by benralizumab

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Asthma affects to 5-10% of population worldwide, estimating that 40-60% of asthmatics have eosinophilic airway inflammation. It is associated with severe eosinophilic asthma, increasing the risk of severe exacerbations, clinical worsening and poor control[1]. Eosinophils play a key role in asthma and containing cytotoxic granule proteins, including major basic protein (MBP) and eosinophil cationic protein (ECP).

Currently, existing different therapeutic strategies for the maintenance of asthma. Benralizumab is indicated as additional maintenance treatment in adult patients with severe uncontrolled eosinophilic asthma, despite administration of high inhaled corticosteroids dosages and long-acting β2-agonists [2]. Benralizumabis a humanized IgG1κ, afucosylated, monoclonal antibody that binds to the IL-5 receptor α on the surface of human eosinophils and basophils[3]. It induces a rapid and complete depletion of blood eosinophils through antibody-dependent cell-mediated cytotoxicity (ADCC), reducing the number of exacerbations and improving lung functionality of severe asthmatics. Indeed, eosinophil apoptosis is induced by proapoptotic proteins, avoiding the eosinophil-degranulation and the secretion of granule-proteins.

Previous studies have shown adverse reactions to benralizumab such as headaches and pharyngitis and, less frequently, hypersensitivity reactions mostly as immediate reactions appearing in the first two hours after administration [4].

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Here, we describe the first case of a patient who suffered a reaction to benralizumabcaused by an unusual secretion of eosinophil mediators immediately after the first drug-dose administration. The patient is a 51-year-old female diagnosed from severe eosinophilic asthma since 1996 with at least four asthma-exacerbations/year, and she is in control for her asthma since 2006 based on a basal spirometry with FEV_1/FVC ratio of 69%, and a positive bronchodilator test of 18% of FEV_1. Four chest radiographs have been done and all were normal. The determination for anti-neutrophil cytoplasmic antibodies and anti-myeloperoxidase has been repeatedly negative. She is not obese, and diffusion and pletismography tests were normal. She was treated with omalizumab without improvement on asthma control, showing persistent obstruction (FEV_1/FVC<70%) and low FEV_1 (<70%) since 2016. Other diagnosis has been discarded following recommendations of the Global Initiative of Asthma (GINA)[5].On the day that she received her first dose of benralizumab, she did not present any flu-like symptoms, she was negative for SARS-CoV-2 and her vitals were the following:90 beats per minute [bpm]; blood pressure [BP]: 115/78mmHg; and peripheral O_2: 94%. First hour later from benralizumab administration she started with chills, nausea, myalgias and general discomfort with 112bpm, body temperature of 37.4ºC and 136/96mmHg. Paracetamol 1g was administered orally. She remained in observation for the next three hours, were fever of 38.6ºC was witnessed and tachycardia persisted; BP and O_2 continued stable. Four hours later, she was discharge with all symptoms resolved and improving vitals (body temperature: 37.80ºC; 104bpm; BP: 109/63mmHg; and peripheral O_2: 94%). Successive benralizumab-doses were well tolerated by the patient with a good clinical response, discarding subsequent reactions to benralizumab or its components. Also, the low number of peripheral eosinophils suggests that the secretion of eosinophil products did not reproduce.

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To study the causes of the clinical features induced by benralizumab, we evaluated several cytokines (IL-10, IL-33, transforming growth factor-beta 1 [TGF-β1], thymic stromal lymphopoietin [TSLP] and periostin) and eosinophil granule proteins (ECP and MBP) in serum before and after benralizumab injection by ELISA. Also, hematological and biochemical parameters were evaluated just before benralizumab-dose and two hours after drug injection (Supplementary Table 1). All detailed methods can be consulted on Supplement Material. The study followed the guidelines set by the Helsinki declaration and was approved by Ethics Committee. Patient was properly advised in writing and provided signed informed consent.

On the one hand, laboratory data collected before the first benralizumab-doseshowed high values of leukocytes, eosinophils and total IgE (Supplementary Table 1). Two hours later, peripheral blood eosinophils and the rest of white blood cells drastically decreased, and total serum IgE increased its values (Supplementary Table 1), similarly to other hypereosinophilic syndrome treated with benralizumab[6].Tryptase, B-factor and complement proteins were normal before and after dose-administration, except for complement C2 protein that reaching values higher of normal range after benralizumab-dose (Supplementary Table 1).

Regarding to cytokines and eosinophil-derived mediators in serum, we observed that ECP and MBP were increased more than two-fold after benralizumab administration (36.23±6.55 vs. 86.16±17.94ng/mL; 34.06±7.60 vs. 77.80±14.97, Figure 1). Similarly, IL-10 was higher after drug injection (non-detectable vs. 0.11ng/mL, Figure 1). On the contrary, we observed a reduction in serum periostin concentration (392.47 vs. 270.09ng/mL, Figure 1) and a slight decreased in IL-33 levels (66.73 vs. 44.38ng/mL, Figure 1) after benralizumab-dose. The rest of measured cytokines did not change (TSLP, Figure 1) or were undetectable (TGF-β1, data not shown).

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According the current literature and the high abnormal values of peripheral blood eosinophils, the symptoms presented by our patients could be produced by adverse effects (AEs) of drug administration, similarly found in hypereosinophilic conditions treated with benralizumab[6]. In a recent clinical phase 2 trial developed by Kuang et al., which included symptomatic adults with a platelet-derived growth factor receptor alpha (PDGFRA)-negative hypereosinophilic syndrome, they reported several AEs including fever, chills, headache, nausea, and fatigue, approximately six hours after the first dose of benralizumab, but not in subsequent doses[6], similarly to our patient.

Although benralizumab benefits and safety for uncontrolled severe eosinophilic asthma have been demonstrated in several previous studies[7], there are still many unanswered questions regarding their behavior in patients with rare diseases after long-term benralizumab therapy. Indeed, some studies have described AEs during benralizumab treatment in eosinophilic asthma,such asLiu et al. in 2019who performed a systematic review of all studies that describe AEs associated to benralizumab on severe asthmatics[8].

Reviewing literature regarding AEs with benralizumab, we believe this could be a case of adverse reaction to benralizumab presumably explained by eosinophil lysis, probablycaused by an eosinophil-degranulation and secretion of eosinophil cationic proteins. Another possibility is that the amount of eosinophil apoptosis was extremely high and secondary necrosis occurred because of limited phagocytic capacity. According to benralizumab pharmacodynamics, the European Medicine Agency stablishes the rapid, near complete depletion of blood eosinophils by cell apoptosis no associated with the increase of ECP and eosinophil-derived neurotoxin (EDN)[9].Indeed, no evidence suggests that benralizumab could induce eosinophil activation. However, we observed an unexpected increase of ECP and MBP in serum

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two hours post-benralizumab. The increase in serum of these cytotoxic proteins is a marker of eosinophil activation and degranulation, being associated to inflammatory processes[10].Likewise, IL-10 have been described among eosinophilic secretory products[11].

In summary, we report a case of a patient with an immediate adverse reaction to benralizumab that suggests a secretion of eosinophil mediators. Although benralizumab has sometimes AEs and do not induce eosinophil-activation, the most probable cause of the clinical features observed was produced by eosinophil-degranulation with secretion of eosinophil-derived proteins.

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Conflict of Interest

JS reports having served as a consultant to Thermofisher, MEDA, Novartis, Sanofi, Leti, FaesFarma, Mundipharma, and GSK; having been paid lecture fees by Novartis, GSK, Stallergenes, Leti, and FaesFarma; as well as having received grant support for research from Thermofisher, Sanofi, and ALK. MJRN reports receiving a grant support for research from Astra Zeneca and GSK, to serve as a consultant to Astra Zeneca and GSK and to have received payments for lectures by Astra Zeneca and GSK. VdP reports

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Figure Legend

Figure 1. Serum protein before and after benralizumab administration. Eosinophil-derived proteins (ECP and MBP) post-benralizumabwere augmented in serum.After benralizumab injection IL-10 was increased and other cytokines were reduced (IL-33, periostin) or did not change their levels (TSLP) in serum. Protein concentration was determined by ELISA. *ECP*, eosinophil cationic protein; *IL*, interleukin; *MBP*, major basic protein; *TSLP*, thymic stromal *lymphopoietin; UND, undetectable*.