

SUPPLEMENTARY MATERIAL

Material and methods

Basophil activation test (BAT; BasoFlowExExbio, Prague, CzechRepublic) was performed with the patient's blood sample and the blood from a non-atopic healthy control following the instructions provided by the manufacturer of the kit. Both the blood from the control and the blood from the patient received the same experimental treatments (incubations with positive controls, and with the tested drugs but undiluted and in serial 1:10 dilutions, as described). Briefly, 100µL of whole blood collected in heparin tubes was treated with 100µL of stimulation buffer for all conditions tested. The negative control condition was left untreated, while the positive control tube was treated with 10µL of a cross-linking anti-IgE antibody mixed with a stimulating peptide, N-formyl-Met-Leu-Phe (fMLP). The rest of tubes were treated with 10µL of the drugs, starting at undiluted concentration, and following by dose-response curves made by serial 1:10 dilutions in PBS solution of each compound. Blood cells were then incubated with either the positive control or the drugs for 15 minutes at 37°C in a water bath. Then 20µL of the antibodies (CD203c-PE and CD63-FITC) were added to each tube and incubation at 4°C for 20 minutes was done. After this, red blood cells were lysed in 300µL of lysis solution for 5 minutes, following by incubation with 3ml of double distilled water for ten minutes. After this, cells were centrifuged at 300g or 5 minutes and resuspended in 300µL of FACS flow solution, and processed in a flow cytometer FACS CANTO II (BD Biosciences) and were analyzed by Infinicyt™ software (BD Biosciences). The population of basophils was defined as CD203c^{pos}/SSC^{low} by flow cytometry. The results are expressed as the

percentage of CD63-positive basophils (activated basophils). A result over 5% of basophil activation is considered as positive result in drug allergy testing¹.

References.

1. Santos AF, Alpan O, Hoffmann HJ. Basophil activation test: Mechanisms and considerations for use in clinical trials and clinical practice. *Allergy*. 2021 Aug;76(8):2420-2432.