Immediate moxifloxacin hypersensitivity : is there more than currently meets the eye?

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Abstract

Immediate drug hypersensitivity reactions (IDHR) to moxifloxacin constitute a pathomechanistic conundrum and a diagnostic challenge. Our objective was to study whether simultaneous phenotyping and quantification of histamine release might add to our knowledge about the basophil activation properties of moxifloxacin and constitute a reliable diagnostic aid. Fifteen patients with an IDHR to moxifloxacin and 9 moxifloxacin challenged controls were selected. All had a basophil activation test (BAT) with moxifloxacin. Flow cytometric analysis of basophil responses implied labeling for CD63, CD203c and intracellular histamine. Unlike tolerant challenged controls, basophilic upregulation of CD203c in response to moxifloxacin was observed in 7/15 patients. Only 2 of these 7 patients demonstrated appearance of CD63 and release of histamine. In the remainder 8 patients no basophil responses were demonstrable. In conclusion, immediate hypersensitivity to moxifloxacin might involve mechanisms difficult to capture by traditional CD63/CD203c-based BAT. Deciphering the complexity of quinolone IDHR seems mandatory.

Key words: basophil activation, drug provocation, flow cytometry, Mas-related G-coupled receptor MRGPRX2, moxifloxacin.

Abbreviations

IDHR: immediate drug hypersensitivity reaction

MRGPRX2: mas-related G-coupled receptor member X2

BAT: basophil activation test

IgE: immunoglobulin E

DPT: drug provocation test

PBS: phosphate buffered saline

EDTA: ethylenediaminetetraacetic acid

FMO: fluorescence minus

DAO: diamino oxidase

SCC: side scatter

THIQ: tetrahydroisoquinoline

MC: mast cells

MC_{TC} : connective-type mast cells

Introduction

Moxifloxacin can cause life-threatening immediate drug hypersensitivity reactions (IDHR), not seldom in naive patients (1, 2). Unfortunately, diagnosis of IDHR to moxifloxacin poses a significant challenge, because of the unavailability of drug-specific sIgE assays and the unreliability of skin testing (3, 4).

The applications of basophil activation tests (BAT) in IDHR have been addressed elsewhere (5, 6). BAT for fluoroquinolones seem to exhibit divergent outcomes, especially with respect to the sensitivity of applied readouts. Although fluoroquinolones might to some extent trigger basophil activation with upregulation of CD203c (7-9), this activation seems more difficult to depict by CD63, a marker of anaphylactic degranulation (9-11). Only Aranda *et al* (12), and in a lesser extent also Fernandez *et al* (9), succeeded to demonstrate appearance of CD63 in response to moxifloxacin. The low sensitivity of the test and divergence between CD63 and CD203c create a diagnostic conundrum but also mirrors a gap in our knowledge about the pathomechanisms of immediate moxifloxacin hypersensitivity. Actually, as IDHR to moxifloxacin is frequently observed in naive patients, it is unlikely these hypersensitivity reactions to result from an adaptive immune response with IgE/FcεRI cross-linking. An alternative pathomechanism could involve an innate immune response with activation of connective-type mast cells (MC_{TC}) via the G-protein-coupled receptor X2 (MRGPRX2) (13). This mechanism might go undetected by traditional BAT, as these cell barely express MRGPRX2 (14).

Here we sought to explore whether simultaneous quantification of alterations of CD63/CD203c surface expression and changes of the intracellular histamine content at a single cell level (15, 16), could help to disentangle the effect of moxifloxacin on basophils.

Methods and materials

Study population

A comparative case-control study was performed between 15 patients with a compelling history of a IDHR to moxifloxacin and 9 healthy controls with a negative graded oral drug provocation test (DPT) with moxifloxacin (Avelox®, Bayer SA-NV, Diegem, Belgium; cumulative dose 750 mg (3)) (table 1). All patients had suffered from urticaria/angioedema, bronchospasm, hypotension and/or loss of consciousness within 1 hour after intake of the drug. Possible alternative causes were excluded. Respectively, 10 patients had a grade 3 and 5 a grade 2 reaction (17). In 11 patients onset of the reaction was extremely rapid varying between 2 and 10 minutes. Time elapsed between the reaction and investigations ranged between 2 and 21 months. All participants gave informed consent approved by the local committee.

Basophil activation experiments

Basophil activation experiments were performed as described in (15, 16). Briefly, aliquots of heparinized whole blood where incubated with 12.5, 125, 625 or 1.250 μmol/L of moxifloxacin (Avelox® 400mg/250 mL, Bayer SA-NV). Dilution buffer and anti-IgE (10 μg/mL, clone G7-18; mouse IgG2a; Pharmingen, BD Biosciences, Erembodegem, Belgium) served as a negative and positive control, respectively. Reactions were stopped by chilling on ice, adding ice-cooled PBS-EDTA 10 mmol/L EDTA and spinning. To select and quantify basophil activation, cells were stained with anti-human IgE (clone GE-1, Sigma Aldrich GmBH, Steinheim, Germany) labeled with Alexa-Fluor 405 (Molecular Probes, Invitrogen, Paisley, UK) and anti-human CD63-FITC (clone H5C6, BD Bioscience), CD203c-APC (clone NP4D6, BD Bioscience) on ice. Cells were lysed/fixed with Phosflow Lyse/Fix buffer (BD Biosciences) and subsequently were washed

twice with PBS with 0.1% Triton-X-100 (PBS-TX, pH=7.4). To stain intracellular histamine, V500-labeled DAO (BD Biosciences) was added and incubated at 37°C (45 min). Cells were washed and re-suspended with PBS with sodium azide and measured.

Flow Cytometric Analysis

Flow cytometric analysis was performed on a FACSCanto II flow cytometer (BD Immunocytometry Systems, San Jose, CA) (15). Correct compensation settings for these fluorochromes were performed using BD CompBeads (BD Biosciences, Erembodegem, Belgium). Fluorescence minus one (FMO) and DAO staining with and without permeabilization was used to set a marker between DAO positive and negative cells. Flow cytometric characterization of basophils relied upon a combination of side scatter, anti-IgE and CD203c. Standardization of intracellular histamine content was performed using standardized fluorospheres (SPHERO Ultra Rainbow Calibration particles, Spherotech, Lake Forest, IL) as described by the manufacturer. Results were expressed as %CD63 positive basophils and as the median histamine release per basophil (%MHC). MHC percentage was calculated as the ratio of the difference between median fluorescence intensity (MFI) / cell in non-degranulating ($CD203c^{dim+}$ and $CD63^{-}$) basophils minus the MFI/cell in degranulating ($CD203c^{hi+}$ and $CD63^{+}$) basophils, against the MFI per cell in non-degranulating ($CD203c^{dim+}$ and $CD63^{-}$) (15).

Statistics

Results are expressed as median (range). The Welch's t-test was applied. Differences were considered significant at a P value < 0.05. Two-graph receiver operating characteristics curve (TG-ROC) analyses was performed to calculate the optimal threshold value and sensitivity/specificity including the corresponding 95% confidence interval (95%CI).

Results

As displayed in figure 1a (left panel), patients but not the challenged control individuals displayed a dose-dependent upregulation of CD203c in response to moxifloxacin. TG-ROC analysis revealed stimulation with 125 µmol/L to be best discriminative between patients and controls. For this stimulation concentration (figure 1a (mid panel)), TG-ROC generated a diagnostic decision threshold value of 16.5% net CD203c upregulation. For this threshold the CD203c-based BAT was positive in 7/15 patients (sensitivity 46.7% (95%CI 25-70%) but none of the 9 challenged controls (specificity 100%). Figure 1a (right panel) displays the individual percentages of CD203c upregulation. Note that 5/7 (95%CI:35-92%) patients with a positive CD203c-BAT had a severe grade 3 reaction. In only 2/15 patients (13.3%; 95%CI: 2-39%) moxifloxacin induced anaphylactic degranulation with appearance of CD63 and decrease of intracellular histamine (figure 1b E,F). As displayed in the right panel of figure 1a, the patients who had a positive CD63 BAT also demonstrated highest CD203c upregulation (squares). Because appearance of CD63 and release of histamine was restricted to 2 patients, no dose-finding nor TG-ROC analysis for these readouts was performed.

Discussion

In the absence of a drug-specific IgE assay and uncertainties associated with moxifloxacin skin testing (3), basophil activation experiments seem an attractive asset to diagnose IDHR to this fluoroquinolone. However, it appears that CD63 and CD203c-based fluoroquinolone BAT might yield divergent results warranting further exploration (7, 8, 10-12). Our triple labelling experiments reveal some intriguing particularities. First, the overall diagnostic sensitivity of the BAT in immediate moxifloxacin hypersensitivity is low, with less than half of the patients responding in the CD203c-BAT. Second, in line with the observations of Fernandez et al (9), moxifloxacin induced an upregulation of CD203c that is generally not accompanied with an

appearance of CD63, a marker that witnesses anaphylactic degranulation (18). Third, as already observed in triple staining experiments in birch pollen allergy (15) and rocuronium hypersensitivity (16), histamine release is restricted to patients demonstrating anaphylactic degranulation.

The exact reason(s) for the low sensitivity and dissociation between CD203c and CD63/histamine release remain(s) elusive but could result from the fluoroquinolone scene to have its own peculiarities, *i.e.* patients with IDHR to fluoroquinolones are frequently drug-naïve (1, 2) and do frequently not show IgE reactivity (12, 19), suggesting an innate rather than an adaptive immune response translated in a new endotype. Alternatively, individuals tolerant to fluoroquinolones frequently demonstrate an unexplained skin test reactivity, probably as result of non-specific histamine release (3, 4).

We hypothesize that moxifloxacin, bearing a tetrahydroisoquinoline motif, might trigger basophils and/or MC through mechanistically different activation pathways that evoke distinct degranulation dynamics and profiles. Based upon the discoveries of McNeil *et al* (13) it is likely that in some patients the clinical manifestations of an immediate moxifloxacin hypersensitivity could have resulted from activation of MC_{TC} through occupation of MRGPRX2 (13). In these patients the reaction could go almost undetected in traditional CD63/CD203c-based BAT, as, unlike MC_{TC}, resting basophils only barely express MRGPRX2 (14). Moreover, the extremely rapid onset of the clinical reactions aligns with the observation that MRGPRX2-triggered degranulation is characterized by a nearly immediate emptying of many individual secretory granules with release of mediators (20). Conversely, we suspect that in some patients the clinical reaction might (also) have resulted from an IgE/FcεRI-mediated anaphylactic degranulation of the effector cells or mixed endotype, mechanisms supposed to be

demonstrable in a traditional CD63-based BAT (18). Alternatively, it cannot be excluded basophils of some of our patients to demonstrate a constitutive or temporarily upregulated expression of the MRGPRX2 receptor (14). Moreover, a basophilic expression could help to explain the dissociation between upregulation of CD203c and non-appearance of CD63 in some patients. In this model engagement of a few MRGPRX2-receptors on the basophil would be insufficient to trigger anaphylactic degranulation of the cells.

In essence, we demonstrate that traditional basophil activation experiments are too insensitive to diagnose immediate moxifloxacin hypersensitivity. The exact reason beyond this poor sensitivity has probably to be sought in the presence different endotypes of phenotypically identical reactions. Moxifloxacin mainly elicits innate immune response with MRGPRX2-dependent degranulation of effector cells. Only the minority of patients who suffered FcεRI-triggered degranulation would be detectable in classical BAT. Additional mechanistic studies are mandatory to validate or refute this speculative but attractive hypothesis, closing the gap in our knowledge about IDHR to this fluoroquinolone, and confirm the potential of BAT in helping to reveal and discriminate mechanistic endotypes. Ideally, such studies should also involve other compounds that trigger MRGPRX2-related effector cell activation.

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Author contributions

Van Gasse Athina L participated in interpretation of data, literature search, data analysis, writing; Sabato Vito participated in interpretation of data, literature search and proofreading final text; Uyttebroek AP participated in interpretation of data and proofreading final text; Elst Jessy participated in proofreading final text; Faber Margaretha participated in proofreading final text; Hagendorens Margo M participated in proofreading final text; Mertens Christel participated in interpretation of data and proofreading final text; Bridts Chris H participated in interpretation of data and proofreading final text; De Clerck Luc S participated in proofreading final text; Ebo Didier G participated in interpretation of data, literature search, data analysis, writing and proofreading final text.

Conflict of Interest Statement

The authors report no conflict of interest.

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