Rocuronium hypersensitivity : does off-target occupation of the MRGPRX2 receptor play a role?

Rocuronium hypersensitivity: Dose MRGPRX2 receptor blockade play a role?

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Abbreviations:

BAT: basophil activation test

FcεRI: high affinity receptor for sIgE

IDT: intradermal test

MC: mast cell

MCA^{+}: mast cell activation

MCA^{-}: no mast cell activation

ROC^{ST+}: positive skin test rocuronium

ROC^{ST-}: negative skin test rocuronium

ROC^{BAT+}: positive BAT rocuronium

ROC^{BAT-}: negative BAT rocuronium

ROC^{BATNR}: non-responder in BAT rocuronium

NMBA: neuromuscular blocking agent

NPV: negative predictive value

POH: perioperative hypersensitivity

PPV: positive predictive value

sIgE: specific IgE antibody

SPT: skin prick test

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Abstract

Background: the neuromuscular blocking agent (NMBA) rocuronium is a relevant cause of perioperative hypersensitivity (POH) with significant risk of diagnostic error. Recently it has been suggested to reclassify hypersensitivity to NMBA as type A reactions resulting from off-target occupation of the nonimmune MRGPRX2 receptor.

Aim: to investigate whether basophil activation experiments can benefit diagnosis and add to the insights in the pathomechanisms of rocuronium hypersensitivity.

Methods: 140 patients with a suspected POH to rocuronium in whom peak tryptase was available had complete diagnostic work-up for all potential culprits including triple confirmatory testing with skin tests, basophil activation (BAT) and quantification of specific IgE antibodies (sIgE) to rocuronium and morphine. To further analyse clinical relevance of sIgE antibodies, quantitative basophil inhibition experiments were performed by coincubation of the cells with rocuronium and morphine, an opiate known to harbour a substituted ammonium structure.

Results: Diagnosis of rocuronium hypersensitivity was established in 72/140 patients (51.4%), of whom 65 (90.3%) demonstrated mast cell activation . Of the 72 patients, 64 displayed a positive skin test, 8 (11.1%) had their diagnosis documented only by BAT. Coincubation of morphine and rocuronium induced a dose-dependent inhibition of basophilic activation with rocuronium that was restricted to 4 out of 6 patients with IgE reactivity to rocuronium and/or morphine.

Conclusion: BAT can benefit diagnosis of rocuronium hypersensitivity. As basophils barely express MRGPRX2 and BAT rocuronium can be inhibited by morphine, we believe hypersensitivity to rocuronium still mainly to result from IgE/FcεRI-dependent effector cell activation. However, it cannot be excluded, in a few patients rocuronium hypersensitivity to result from off-target occupation of the MRGPRX2 receptor.

Highlights Box

What is already known about this topic

The neuromuscular blocking agent rocuronium is a relevant cause of perioperative hypersensitivity. Recently it has been suggested to reclassify hypersensitivity to NMBA as type A reactions resulting from off-target occupation of the nonimmune MRGPRX2 receptor.

What does this article add to our knowledge?

According to the results of triple testing (skin tests, basophil activation, quantification of sIgE) and quantitative basophil inhibition experiments we think hypersensitivity to rocuronium mainly to result from a genuine IgE/FcεRI-dependent effector cell activation.

How does this study impact current management guidelines?

We think hypersensitivity to rocuronium still mainly to result from IgE/FcεRI-dependent effector cell activation. However, it cannot be excluded, in some patients rocuronium hypersensitivity to result from off-target occupation of the MRGPRX2 receptor.

Introduction

Rocuronium is an aminosteroid non-depolarizing neuromuscular blocking agent (NMBA). Different surveys regarding perioperative hypersensitivity (POH) indicate that rocuronium is a relevant - and probably still increasing - cause of severe POH [1, 2]. In order to optimize correct diagnosis of rocuronium hypersensitivity and to reduce diagnostic errors we recently published a diagnostic algorithm in the Journal [3]. This algorithm shows that skin tests still merit the status of primary diagnostic investigation with a positive predictive value (PPV) of 98% and a negative predictive value (NPV) of 96% [4]. Alternatively, it is clear that the diagnosis of rocuronium hypersensitivity should not solely rest upon a positive result for rocuronium-specific IgE (sIgE) nor sIgE to morphine [5]. These sIgE tests show too low specificity [4, 5], mainly because of nonspecific binding to the solid phase as observed with elevated total IgE titres [6].

At present 10 studies have investigated the diagnostic performances of basophil activation tests (BAT) in NMBA hypersensitivity [3]. Compared to skin testing traditional BAT attains a sensitivity between 36-92% and specificity between 81-100%. For rocuronium, the BAT reaches an excellent PPV of 97% and an NPV of 75% [4]. However, to our knowledge, there are currently no data on the added value of BAT in patients displaying equivocal or negative skin tests.

Here we sought to investigate whether BAT rocuronium could benefit diagnosis and identify patients in whom diagnosis would have been overlooked because of negative skin test investigation. Furthermore, we hypothesize on the potential of BAT and basophil coincubation experiments to further clarify on the proposal to reclassify NMBA hypersensitivity as an IgE/FcεRI-independent reaction resulting from off-target occupation of the nonimmune receptor MRGPRX2 [7].

Materials and methods

Study population

We evaluated a total of 140 patients who were referred to our outpatients' clinic for diagnostic evaluation after experiencing a POH reaction grade 1-4 according to the National Audit Project (NAP6) severity criteria [8]. All had rocuronium as NMBA and quantification of peak serum tryptase within 90 minutes after onset of their reaction. All patients underwent a standardized protocol for all potential offenders of perioperative anaphylaxis [9]. With respect to rocuronium, all patients underwent skin testing, BAT and quantification of sIgE to rocuronium but also sIgE morphine [4], the latter being a marker for sensitization to tertiary and quaternary substituted ammonium structures [6, 10, 11].

Confirmatory testing for rocuronium

All individuals had skin tests with the aminosteroid (rocuronium, Esmeron®, Merck Sharp and Dohme, Brussels, Belgium), negative control (saline buffer), and a positive control (10 mg/mL histamine; HAL

Allergy Benelux NV, Haarlem, the Netherlands). Skin tests included skin prick tests (SPT) and, if negative, intradermal tests (IDT). Maximal test concentration was 10 mg/mL (undiluted) for SPT and 0.05 mg/mL (dilution 1/200) for IDT [12]. For the SPT and the IDT, rocuronium was diluted immediately before use. SPT with a wheal $\geq$ 3 mm with surrounding erythema after 15 min were considered positive. For IDT, injection of 0.05 mL was performed and reactions were read after 20-30 min. IDT responses with a wheal and flare $\geq$ 8 mm (or doubling of injection bleb) were considered positive.

The BAT for rocuronium is described in detail elsewhere [13]. Results were expressed as the net percentage of CD63+ basophils and threshold of positivity was set at 4% [13].

Specific IgE to rocuronium and morphine was quantified by ImmunoCAP system (Phadia Thermo Fisher, Uppsala, Sweden) according to the manufacturer's instructions. For rocuronium decision threshold was set at 0.13 kU_A/L and for morphine at 0.35 kU_A/L [6].

To elucidate on the clinical relevance of sIgE rocuronium antibodies, in a separate set of experiments, we compared the effect of basophilic coincubation with morphine and anti-IgE (positive control) as well as morphine and rocuronium between 6 patients with positive sIgE results and 3 patients with negative sIgE results to rocuronium and/or morphine.

Mast cell activation (MCA) was defined as a peak value exceeding 1.2xbaseline tryptase + 2, as recently validated in POH [14].

Results

Figure 1 displays the different patients' groups according to the presence or absence of MCA and outcomes of skin testing and BAT. In total, 140 rocuronium-exposed patients who experienced an POH reaction grade 1 - 4 underwent a standardized diagnostic protocol for all potential culprits. All diagnostics were performed between 7 and 3672 days after the index reaction. One hundred and six cases had displayed MCA (MCA^+), whereas 34 did not (MCA^-).

From the 106 MCA^+ patients, 57 had a positive skin test to rocuronium (MCA^+ROC^{ST+}), 49 had a negative skin test for rocuronium (MCA^+ROC^{ST-}). From the 57 MCA^+ROC^{ST+} patients 30 displayed a positive BAT for rocuronium (52.6%, $MCA^+ROC^{ST+BAT+}$), 19 a negative BAT rocuronium ($MCA^+ROC^{ST+BAT-}$) and 8 were non-responders to stimulation with both the positive control anti-IgE and the drug ($MCA^+ROC^{ST+BATNR}$). From the 49 MCA^+ROC^{ST-} patients, 8 had a positive BAT for rocuronium ($MCA^+ROC^{ST-BAT+}$). The details of these 8 $MCA^+ROC^{ST-BAT+}$ patients are summarized in table 1. Thirty MCA^+ROC^{ST-} patients had a negative BAT ($MCA^+ROC^{ST-BAT-}$), and 11 were non-responsive to positive control stimulation ($MCA^+ROC^{ST-BATNR}$). In the 8 $MCA^+ROC^{ST-BAT+}$ patients no other cause was identifiable, suggesting a causative role for rocuronium. In contrast, in all of the 11 non-responders and 19 of the 30 $MCA^+ROC^{ST-BAT-}$ patients another culprit was identified, mainly the β-lactam antibiotic cefazolin and chlorhexidine. Only 2 out

of the 11 $MCA^{+}ROC^{ST-BAT-}$ who had no clear diagnosis established demonstrated sensitization to substituted tertiary and quaternary ammonium structures, as indicated by a sIgE to morphine of 7.39 kU_A/L and 0.47 kU_A/L. In these patients total IgE was 304 kU/L and 21 kU/L, respectively.

From the 34 patients without MCA (MCA^{-}), 7 had a positive skin test for rocuronium ($MCA^{-}ROC^{ST+}$) of which only 1 (14.3%) had a positive BAT ($MCA^{-}ROC^{ST+BAT+}$) and 3 demonstrated a positive sIgE to morphine. The remainder 6 $MCA^{-}ROC^{ST+}$, all responsive to positive control stimulation, had a negative BAT rocuronium ($MCA^{-}ROC^{ST+BAT-}$). All 27 $MCA^{-}ROC^{ST-}$ patients also had a negative BAT for rocuronium ($MCA^{-}ROC^{ST-BAT-}$). In 20 out of these 27 $MCA^{-}ROC^{ST-BAT-}$ patients no cause was delineable. Two of these 20 $MCA^{-}ROC^{ST-BAT-}$ patients without demonstrable cause demonstrated sensitization to substituted tertiary and quaternary ammonium structures, as indicated by a sIgE to morphine of 0.87 kU_A/L and 0.61 kU_A/L. In these patients total IgE was 50 kU/L and 193 kU/L, respectively. Figure 1 also displays the number of positive sIgE results in the different patients' groups.

Overall, 72/140 patients (51.4%) were diagnosed as hypersensitive to rocuronium of whom 8 (11.1%) had negative skin tests and their diagnosis documented by the BAT (see table for individual results). Alternatively, a total of 25 ROC^{ST+} patients with responding basophils displayed a negative BAT, with 15 (60%) demonstrating a positive sIgE to morphine (12 MCA^{+}, 3 MCA^{-}).

Table 2 and figure 2 summarize the demographics, biological findings and the results of the basophil coincubation experiments. Coincubation of morphine and rocuronium induced a significant dose-dependent inhibition of basophilic activation with rocuronium that was restricted to 4 of the 6 patients with IgE reactivity to rocuronium and/or morphine. No effect was demonstrable in the patients with negative sIgE results. No effect of morphine was demonstrable on the BAT with anti-IgE.

Discussion

To our knowledge this is the first study to endorse the complementary value of the BAT in the diagnosis of rocuronium hypersensitivity. From our results it emerges that, although hypersensitivity to rocuronium is more prevalent in the MCA^+ group, it is certainly not restricted to patients who demonstrate MCA. As a consequence, diagnostic work-up with confirmatory testing should not be restricted to the MCA^+ group but also involve the MCA^- group. In a majority of our patients diagnosis of rocuronium hypersensitivity is established by skin testing, confirming this diagnostic procedure merits the status of primary diagnostic approach [3]. However, 11% of our patients demonstrate negative skin test responses and have their diagnosis of rocuronium hypersensitivity finally established by BAT (and most also by sIgE). Although, for obvious reasons, it is impossible to perform full-dose challenges with this NMBA, we are confident the positive BAT results in our skin test negative patients are clinically significant and indicate a genuine sIgE/FcεRI-dependent rocuronium hypersensitivity. As a matter of fact, as already exemplified in the introductory paragraph, the BAT rocuronium has an excellent PPV [4] and has been integrated in the diagnostic approach of rocuronium hypersensitivity in patients who display negative or equivocal skin test results [3].

Alternatively, it is confirmed that BAT can be negative in ROC^{ST+} patients. Although it is likely negative BAT outcomes mainly to result from a non-responder status of the cells or a lower test sensitivity of BAT, it is not excluded that the $ROC^{ST+BAT-}$ status of some patients might have an alternative explanation. Actually, in ROC^{ST+} patients negative BAT results might relate to the existence of an alternative pathomechanism of rocuronium hypersensitivity that is independent from IgE/FcεRI cross-linking [7]. In 2015 McNeil *et al* [15], demonstrated that engagement of the Mas-related G-protein receptor MRGPRX2 might be implicated in mast cell (MC)-driven immediate drug hypersensitivity reactions (IDHR) that are phenotypically indistinguishable from IgE/FcεRI-dependent MC degranulation. Potential MRGPRX2 agonists identified in this study are the bradykinin receptor 2 antagonist icatibant, NMBA (atracurium, rocuronium), and several fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin). Recently similar observations were made for opioid compounds (including morphine, codeine and different major metabolites) [16], vancomycin [17] and many other antimicrobials [18]. The quintessence of these studies is clear, *i.e.* off-target occupancy of the MRGPRX2 receptor is increasingly recognized as a novel nonimmune endotype of MC-driven IDHR. Moreover, the cellular distribution of the MRGPRX2 might explain the discrepancies between positive skin test responses and negative outcomes of BAT in our patients. As a matter of fact, as basophils, unlike cutaneous $MC_{TC,}$ barely express the MRGPRX2 on their surface [19], and because basophils do not respond non-specifically to rocuronium in individuals uneventfully exposed to this NMBA [4, 5, 13], one could, like for opiates [20] and fluoroquinolones [21], speculate basophil activation experiments not only to constitute a diagnostic to

document genuine IgE/FcεRI-mediated rocuronium hypersensitivity but also to be an interesting asset to further explore the putative existence of an MRGPRX2-related endotype. This particular nonimmune endotype, would not only be independent of IgE/FcεRI-cross-linking but also go undetected in steady state conditions of traditional CD63-based BAT. To verify the hypothesis that noncongruent positive skin tests and negative BAT might reflect an IgE/FcεRI-independent endotype we firstly compared sIgE to both rocuronium and morphine (the "marker" for sensitization to tertiary and quaternary ammonium structures) between $ROC^{ST+BAT+}$ and $ROC^{ST+BAT-}$ patients who exhibited MCA during their POH reaction. Secondly, we performed basophil coincubation experiments with the positive control (anti-IgE) and morphine as well as rocuronium and morphine. A dose-dependent inhibition of the BAT rocuronium by morphine would be indicative for clinical relevance of sIgE rocuronium. Although no absolute conclusions can be drawn, the positive results for sIgE in about two-thirds of the patients in both groups, and the observation morphine to significantly inhibit BAT rocuronium in most patients with positive sIgE results call for prudence on the proposal rocuronium mainly to cause IgE/FcεRI-independent hypersensitivity and to reclassify these reactions as nonimmune type A reactions [7]. Such a reclassification would refute triple positive testing (skin test, BAT, sIgE) as a proof of IgE/FcεRI-dependent hypersensitivity. Moreover, a MRGPRX2-dependent mechanism is also difficult to reconcile with the observation 11% of patients demonstrating negative skin tests but a positive BAT. *Mutatis mutandis* we call for restraint on a reclassification of hypersensitivity to other NMBA as nonimmune type A reaction. For example, for atracurium it has also been demonstrated that BAT [22-25] and quantification of sIgE to atracurium [10, 26, 27] can benefit diagnosis. Moreover, evidence has accumulated atracurium sIgE antibodies to exhibit other specificities than sIgE antibodies to rocuronium and suxamethonium [10, 26-28]. Collectively, we believe NMBA mainly to cause IgE/FcεRI-dependent hypersensitivity but it is tempting to speculate that (some of) these drugs, in some patients, might trigger MC degranulation, by off-target occupancy of the MRGPRX2 receptor. Whether this alternative explanation applies to rocuronium remains elusive. Although significant progress has been made in our knowledge about NMBA-induced MRGPRX2-dependent MC degranulation, our insights for rocuronium remain incomplete and uncertain. Actually, Lansu *et al* [16], although using a similar calcium imaging technique, could not confirm rocuronium to be a secretagogue agonist for MRGPRX2 in a LAD2 human MC line, as proposed by a mouse model used by McNeil *et al* [15]. The explanation for the divergences between mice and human MC has probably to be sought in adaptive changes of the MRGPRX2 gene in human evolution [29], making the human receptor more than a 10-fold less susceptible for rocuronium than its murine orthologue Mrgprb2 [15]. It is of note that in the recent NAP6 survey, only 4 patients who reacted to a benzylisoquinoline NMBA and none of the patients who reacted to rocuronium were classified by the authors as "non-allergic" [30], as all demonstrated positive skin test

and/or sIgE results [8]. Finally, 6 years after pholcodine withdrawal, the Norwegian population has become less IgE-sensitized and clinically more tolerant to NMBA [31].

In conclusion, it is clear that rocuronium hypersensitivity cannot mainly be attributed to off-target occupancy of the MRGPRX2 receptor and that basophils and sIgE antibodies do still matter. First, the BAT is complementary to skin testing to document rocuronium hypersensitivity. Second, we believe that congruent positive triple testing (skin test, BAT and sIgE) and the quantitative basophil inhibition data, favour rocuronium hypersensitivity mainly to result from genuine IgE/FcεRI-dependent effector cell activation. Alternatively, it should be admitted that noncongruent positive skin test and negative BAT and sIgE data could point to the existence of an alternative mechanistic endotype of rocuronium hypersensitivity independent from IgE/FcεRI cross-linking and eventually to occur as a result of off-target occupancy of the MRGPRX2 receptor. However, additional mechanistic studies in human MC and basophils are required to fill the current knowledge gaps and to enable eventual shifting of the IgE/FcεRI – MRGPRX2 paradigm for this NMBA.

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Figure legends

Figure 1: Flowchart displaying presence or absence of mast cell activation (MCA), skin test, basophil activation and sIgE results.

Figure 2: Quantitative inhibition experiments expressed as individual normalized percentages of CD203c++CD63+ BAT (n=9).

The BAT with the positive control anti-IgE is not affected by coincubation with morphine (A).

In contrast, there is a dose-dependent inhibition of BAT rocuronium with morphine (0-400 µmol/L) in 4 out of the 6 patients with positive sIgE to rocuronium and morphine (full lines). In contrast, there is no inhibition of the BAT rocuronium in 3 patients with negative sIgE to rocuronium and morphine (dashed lines) (B).

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