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Original article

Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide

Background: Diagnosis of anaphylaxis from neuromuscular blocking agents (NMBA) is not always straightforward.
Objectives: To assess flow cytometric analysis of activated basophils (BAT) as a diagnostic instrument in anaphylaxis from rocuronium. To investigate whether the technique might help to identify cross-reactive and safe alternative compounds.
Methods: For validation of the BAT, 14 patients with perioperative anaphylaxis demonstrating a positive skin test (ST) for rocuronium and eight individuals that tolerated rocuronium and a negative ST for this drug were enrolled. To confirm specificity of the BAT, five patients that tolerated atracurium or cisatracurium with a negative ST for rocuronium were tested. Basophil activation with rocuronium, vecuronium, atracurium, cisatracurium and suxamethonium was analysed flow cytometrically by labelling with anti-CD123/anti-HLADR/anti-CD63.
Results: Sensitivity of BAT for rocuronium was 91.7% and specificity 100%. However, in two patients the BAT was lost as a diagnostic tool, as their cells were nonresponsive to positive control stimulation and allergen. Seven from the 12 responsive patients also demonstrated a clear basophilic activation for vecuronium. Moreover, according to ST and/or BAT cross-reactivity between rocuronium and vecuronium was suspected in 10/14 patients. Except one patient, all patients had negative BAT and ST investigations for atracurium and cisatracurium. Currently, five patients tolerated administration of cisatracurium. All control individuals demonstrated negative ST and BAT for all tested NMBA.
Conclusions: The BAT constitutes a reliable instrument to diagnose anaphylaxis from rocuronium. The technique also allows quick and simultaneous testing of different potential cross-reactive NMBA and to tailor a safe alternative.

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Diagnosis of anaphylaxis from neuromuscular blocking agents (NMBA) is not always straightforward. Therefore, additional reliable diagnostic tools are of tremendous interest.

Currently three studies have assessed flow-assisted diagnosis of allergy from NMBA (1–3), and it appears that the technique constitutes a promising instrument for the diagnosis of hypersensitivity from NMBA. However, additional comprehensive studies are mandatory to further validate the activated basophils (BAT) and allow its entrance in mainstream application [review: (4), Ebo DG et al. unpublished data].

This study assesses the BAT in the diagnosis of anaphylaxis from rocuronium and evaluates whether the BAT could provide an instrument to identify cross-reactive NMBA and to tailor individual safe neuromuscular blocking regimens.

Methods

Study population

Fourteen patients who had presented hypotension and/or bronchospasm within 5 min after injection of rocuronium demonstrating a positive ST for the drug were enrolled. Possible alternative causes (e.g. latex, chlorhexidine, antibiotics, analgesics and hypnotics) were excluded as described in (5). Eight individuals had tolerated rocuronium and demonstrated a negative ST served as a control group (C1). Basophil activation and ST were performed between 6 weeks and 3.5 years after the acute reaction. To confirm

specificity of the BAT, five control individuals who tolerated administration of a benzylisoquinoline (four cisatracurium and one atracurium) and had negative ST for rocuronium (C2) were studied separately.

Skin tests

Patients and control individuals had ST according to (6) with rocuronium (Esmeron®; Organon, Brussels, Belgium), vecuronium (Norcuron®; Organon), atracurium (Tracrium®; GSK, Genval, Belgium), cisatracurium (Nimbex®; GSK), suxamethonium (Myoplegine®; Christiaens, Brussels, Belgium), latex (Stallergènes, Genval, Belgium), chlorhexidine digluconate 2% in 70% alcohol, the administered analgesics and hypnotics, a negative control (phenol-containing buffer; HAL Allergy Benelux BV, Haarlem, the Netherlands) and a positive histamine (10 mg/ml, HAL Allergy Benelux BV) control. Skin prick test (SPT) and intradermal test (IDT) responses were considered positive when the wheal equalled or exceeded a diameter of 3 and 8 mm, respectively. Drugs were diluted in a phenol-containing buffer (HAL Allergy Benelux BV) immediately before use.

Basophil activation test

Within 3 h after sampling in endotoxin-free heparinized tubes (Vacuette, Greiner Labortechnik GmBH, Kremsmünster, Austria), aliquots of 100 µl whole blood were stimulated (20 min, 37°C) with serial dilutions of rocuronium ($0.5–5 \times 10^3$µg/ml), vecuronium (0.5–2×10^3 µg/ml), atracurium ($0.5–5 \times 10^3$ µg/ml), cisatracurium (0.5–10^3 µg/ml) and suxamethonium ($0.5–5 \times 10^3$ µg/ml), 20 µl (10 µg/ml) anti-IgE (Pharmingen BD Biosciences, Erembodegem, Belgium) as a positive control, or 20 µl buffer as a negative control. Dilutions were carried out in an IL-3 containing stimulation buffer (2 ng/ml, BD Biosciences). Adding 10 µl of 20 mM EDTA (5 min, room temperature) stopped the reaction. For flow cytometric quantification (FACSCalibur; BD, Immunocytometry Systems, San Jose, CA, USA) of BAT cells were stained with 20 µl of a mixture containing anti-CD123-PE, anti-human leucocyte antigen DR-PerCP and anti-CD63-FITC conjugated antibodies (Pharmingen BD Biosciences), during 20 min in the dark, on ice. Red blood cells were lysed and white blood cells were fixed (FACS Lysing solution, BD) during 10 min at room temperature. After centrifugation (5 min, 250 ***g***, 4°C) 200 µl of washing solution was added to the cell pellets.

Basophils were selected on a CD123+/HLA-DR- gate and at least 500 basophils were counted. Subsequently, within this gate the percentage of activated basophils, i.e. co-expressing CD63 was measured (Fig. 1). For this purpose, the marker was set on the 99th percentile value of the fluorescein isothiocyanate-conjugated irrelevant control antibody. Results were expressed as the percentage of CD63+ basophils.

Statistical analysis

Results were expressed as median (range). The Mann–Whitney *U*-test was applied. Differences were considered significant at a P value < 0.05. Two-graph receiver operating characteristics (TG-ROC) curve analyses was performed to calculate the optimal cut-off value [and its 95% confidence interval (CI)] corresponding to the best sensitivity and specificity (7).

Results

Skin tests

In 13/14 patients rocuronium anaphylaxis was diagnosed upon SPT. One patient needed additional IDT.

In analogy to others (8–11), as a first measure to assess cross-reactivity between NMBA, patients had additional ST with vecuronium, atracurium, cisatracurium and suxamethonium. A positive ST for vecuronium was found in 7/14 patients, one also showing ST responsiveness for atracurium. Two patients had a positive ST for suxamethonium. All the patients demonstrated a negative ST for cisatracurium. All control individuals demonstrated negative ST responses to the five NMBA tested.

Activated basophils

Two patients were nonresponders to positive control stimulation. As, in these patients it is impossible to

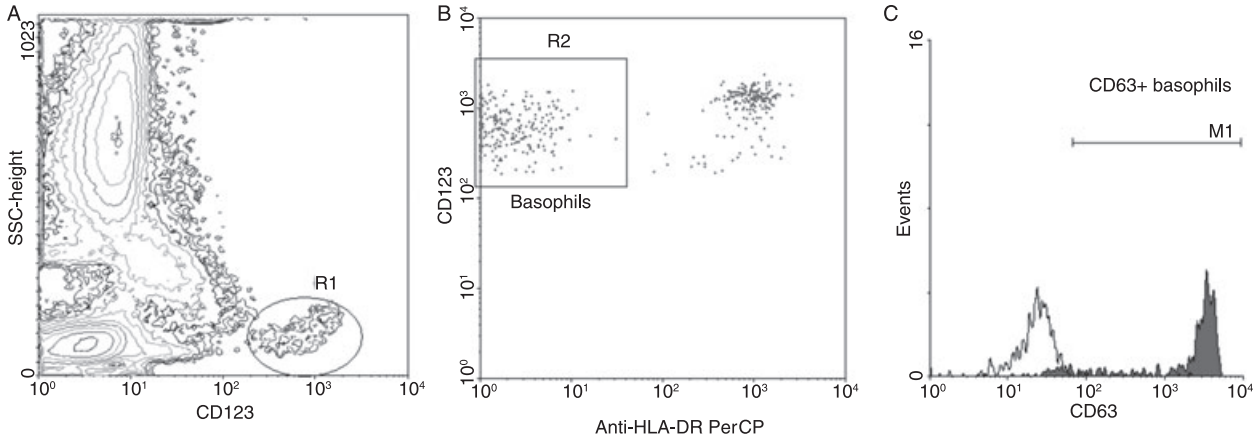

Figure 1. Flow cytometric analysis of basophils selected on: (A) CD123+/SSC gate (R1); (B) CD123+/HLA-DR- gate (R2); (C) CD63 expression after challenge with buffer (open histogram) and rocuronium (closed histogram). Marker M1 denotes CD63 positive cells and was set on fluorescein isothiocyanate-conjugated irrelevant control antibody.

interpret negative drug stimulation, they were withdrawn from further analysis. In the 12 responders spontaneous and anti-IgE-induced CD63 expression was comparable to results in control individuals (data not shown).

Rocuronium. In C1 control individuals, rocuronium-induced CD63 expression remained comparable to spontaneous expression. In contrast, responders demonstrated a clear dose-dependent CD63 up-regulation (Fig. 2A). Two-graph receiver operating characteristics analysis, revealed stimulation with 5×10^2 μg/ml to be most discriminative between patients and C1 controls, and generated a diagnostic threshold value of 4% CD63 up-regulation (95% CI: 1–6%, Fig. 3). For this threshold the BAT was positive in 11/12 responders (sensitivity 91.7%) and 0/8 C1 controls (specificity 100%), respectively. Figure 4 summarizes the individual percentages of CD63 up-regulation and number of positive BAT in responsive patients and control groups.

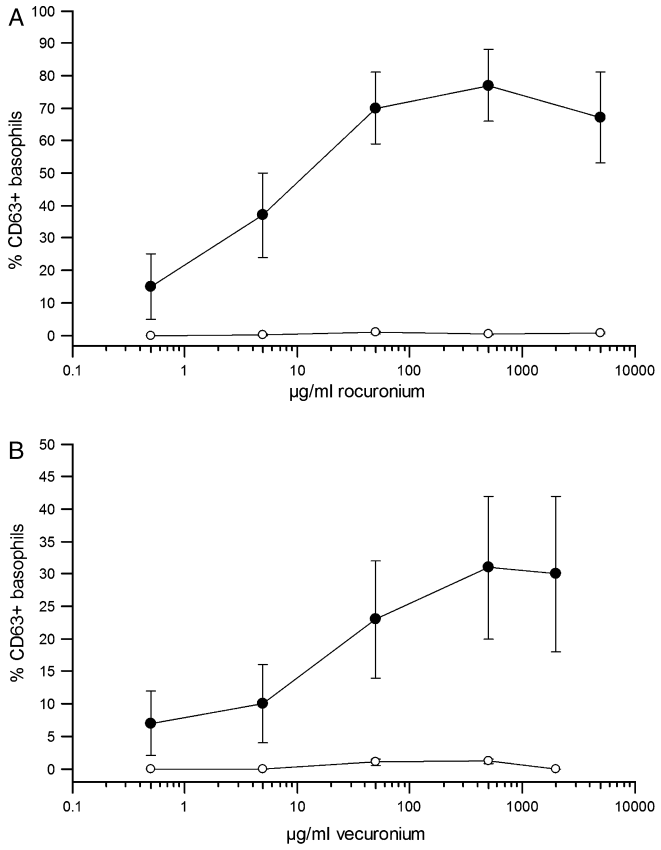

Figure 2. (A) Rocuronium-induced CD63 up-regulation in rocuronium allergic patients ($n = 10$, closed circles) and control individuals that tolerated administration of rocuronium and demonstrated a negative skin test to rocuronium (C1, $n = 8$, open circles). (B) Vecuronium-induced CD63 up-regulation in rocuronium allergic patients ($n = 10$, closed circles) and control individuals that tolerated administration of rocuronium and demonstrated a negative skin test to rocuronium (C1, $n = 8$, open circles). Results are expressed as the percentage CD63 positive basophils (means ± SEM).

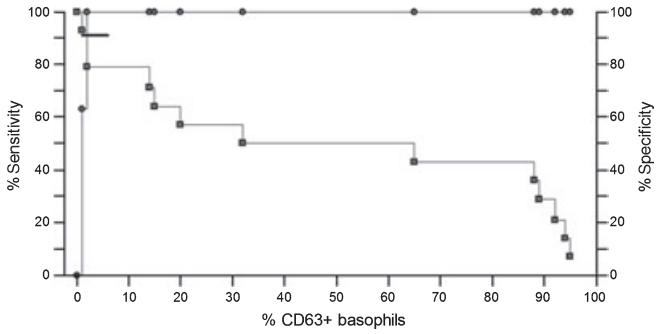

Figure 3. Two-graph ROC curve for rocuronium-induced CD63 expression generated between rocuronium allergic patients ($n = 12$) and control individuals that tolerated administration of rocuronium and demonstrated a negative skin test to rocuronium (C1, $n = 8$). Stimulation concentration of rocuronium: 5×10^2 μg/ml. The bold line denotes the 95% confidence interval that spans from 1% to 6% around an optimal threshold of 4%. Squares represent sensitivity whereas circles represent specificity.

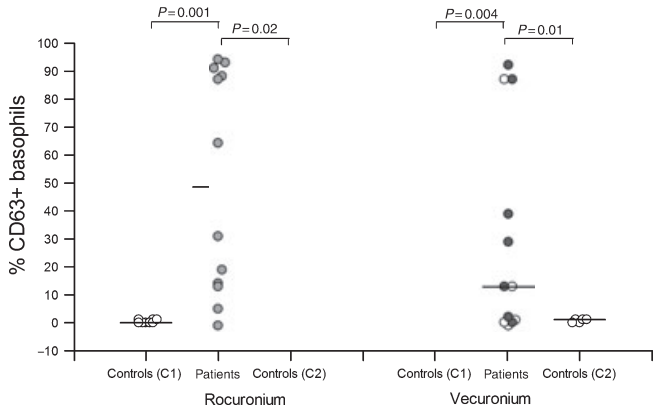

Figure 4. Left: rocuronium-induced CD63 expression in control individuals that tolerated administration of rocuronium and demonstrated a negative skin test to rocuronium (C1), responsive patients allergic to rocuronium, and control individuals who tolerated administration of a benzylisoquinolines-derived neuromuscular blocking agent and demonstrated a negative skin test to rocuronium (C2). Open circles represent negative skin tests with rocuronium. Closed circles represent the positive skin tests with rocuronium. Right: vecuronium-induced CD63 expression in control individuals that tolerated administration of rocuronium and demonstrated a negative skin test to rocuronium (C1), responsive patients allergic to rocuronium, and control individuals that tolerated administration of a benzylisoquinolines-derived neuromuscular blocking agents rocuronium and demonstrated a negative skin test to rocuronium (C2). Open circles represent negative skin tests with vecuronium. Closed circles represent the positive skin tests with vecuronium. Results are expressed as percentages of CD63 positive basophils. The bold lines denote the medians.

Vecuronium. In contrast to C1 controls, patients also demonstrated a dose-dependent up-regulated basophilic CD63 expression with vecuronium (Fig. 2B). According to the most discriminative stimulation of 5×10^2 μg/ml distinct CD63 up-regulation was observed in 7/12 responders (Fig. 4). Most importantly, the BAT and ST proved clearly complementary in identification of cross-reactivity between rocuronium and vecuronium. Actually, five patients were double positive in BAT and ST for vecuronium, three were double negative, two patients demonstrated a positive BAT and negative ST, *vice versa* two patients demonstrated ST reactivity and a negative BAT.

All the benzylisoquinoline tolerant individuals demonstrated a negative BAT for vecuronium.

Suxamethonium and benzylisoquinolines. Apart from two patients that demonstrated basophilic activation with suxamethonium (paralleling a positive ST), no cell activation for suxamethonium, atracurium and cisatracurium was demonstrable in patients and controls. Relative to spontaneous expression, up-regulation of CD63 expression generally varied between 0% and 3%.

Discussion

Our data reveal that rocuronium tolerant individuals showed no rocuronium-induced basophilic activation. In contrast, patients with rocuronium anaphylaxis showed significant rocuronium-induced basophilic activation. However, as addressed by others (1), patients demonstrated different optimal stimulation concentrations of rocuronium. Nevertheless, TG-ROC analysis demonstrated stimulation at 5×10^2 μg/ml to be most discriminative. For this 'optimal' stimulation concentration, the BAT attained a sensitivity of 91.7% and specificity of 100%. However, in two patients with negative drug stimulation, the BAT was lost as a diagnostic tool because of nonresponsiveness to positive control stimulation.

One might argue our threshold to differ considerably from cut-offs values of 10–15% published earlier (1–3). However, some important issues have to be addressed. First, anaphylaxis from NMBA can be life-threatening. Therefore, it is critical to establish a sensitive cut-off. We had the opportunity to include well-characterized rocuronium allergic and tolerant individuals, enabling precise TG-ROC analysis for calculation of the best discriminative threshold. In two former publications threshold values were chosen arbitrarily relative to spontaneous expression (2, 3). Secondly, optimal stimulation conditions might differ considerably from NMBA to NMBA (1), rendering the proposal to apply a single threshold value for all NMBA difficult to justify. Our threshold applies to rocuronium. No conclusions are drawn for other NMBA. Thirdly, it has been pointed out drugs to yield lower percentages of BAT upon stimulation as compared to protein allergens (12, 13). Finally, specificity of our threshold was endorsed in an analysis on control individuals that had tolerated a benzylisoquinoline.

Several investigators have demonstrated cross-reactivity between NMBA to be common (8–11, 14). Cross-reactivity appears to be more prevalent between aminosteroids than between benzylisoquinolines. Therefore, our second objective was to assess whether the BAT could contribute in the identification of potential cross-reactive and/or safe alternative drugs.

Our data re-emphasize evaluation of anaphylaxis from NMBA is not appropriate when it failed to address the possibility of cross-reactivity and did not identify a safe alternative regimen. A clear majority of our patients demonstrated compelling evidence for cross-reactivity between rocuronium and vecuronium. It is noteworthy that, in the assessment of cross-reactivity between NMBA, the BAT and ST appear clearly complementary. The BAT might identify cross-reactivity between rocuronium and vecuronium missed by ST. Alternatively ST picked-up cross-reactivity overlooked by the BAT.

From our data it emerges that benzylisoquinolines generally constitute a safe substitute for aminosteroids in anaphylaxis from rocuronium. Except one patient who demonstrated a positive SPT for atracurium, all patients had negative ST and BAT investigations for benzylisoquinolines. Meanwhile, based upon our assessment, five patients have been administered cisatracurium uneventfully. This parallels the data of Karila et al. (11) who failed to demonstrate cross-reactivity between cisatracurium and vecuronium and the classification of cisatracurium as a NMBA with low potential for anaphylaxis (9).

In conclusion, the BAT constitutes a reliable diagnostic instrument for anaphylaxis from rocuronium. It also provides the physician with a complementary tool that allows simultaneous testing of different potential cross-reactive NMBA and helps to tailor a safe alternative. Once fully validated, this quick (results available within 3 h) technique could rapidly enter daily clinical practice because the numerous flow cytometers already placed in the laboratories.

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