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Flowcytometric diagnosis of atracurium-induced anaphylaxis.

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Abstract

Background: Allergy to atracurium is a rare condition with serious consequences of diagnostic error. However, correct diagnosis is not always straightforward.

Objectives: To assess the utility of the basophil activation test (BAT) in atracurium sensitization and to investigate its role in identifying cross-reactivity between muscle relaxants.

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Methods: For validation 8 patients with perioperative anaphylaxis to atracurium and 7 individuals experiencing perioperative anaphylaxis but not exposed to neuromuscular blocking agents (NMBA) were included. Furthermore, 5 other patient groups were included in the study, all individuals exposed to different NMBA, either sensitized or not to the drug. Basophil activation with atracurium was analysed flow cytometrically.

Results: ROC analyses between 8 atracurium sensitized patients and 7 non-exposed controls allowed identification of 5% as the decision threshold for BAT positivity. For this cut off the BAT attained a sensitivity of 63%, specificity of 100%, positive predictive value of 100% and negative predictive value of 70%. Of the atracurium-exposed individuals with a negative atracurium skin test, 2 individuals had a clear positive BAT. BAT atracurium was positive in one cisatracurium sensitised patient and negative in all cisatracurium exposed patients with a negative skin test to cisatracurium. All rocuronium and suxamethonium-sensitized patients displayed a negative BAT with atracurium.

Conclusions: The BAT proves to be a useful diagnostic for atracurium-induced anaphylaxis and may be complementary to skin tests. The technique enables quick and simultaneous testing of potentially cross-reactive NMBA and the identification of safe alternatives for future surgery.

Introduction

Genuine IgE-mediated atracurium allergy is a rare condition with severe consequences of diagnostic error (1, 2). In the absence of a validated atracurium-specific IgE assay, diagnosis of atracurium allergy and identification of safe alternatives relies almost uniquely upon skin testing and eventually measurement of sIgE towards substituted ammonium compounds such as the opiate morphine (3). However, although at the moment considered to be the gold standard; skin testing to benzylisoquinoline drugs does not demonstrate an absolute predictive value (3, 4). False negative skin tests (ST) might severely endanger the life of our patients, whereas false positive skin tests can lead to overdiagnosis and unnecessary avoidance strategies. Alternatively, the sensitivity of morphine sIgE in benzylisoquinoline sensitization has been reported to vary considerably between 25% (5) and 75% (6). Therefore, additional safe and reliable diagnostics are of tremendous interest.

Upon activation with a specific allergen basophils up-regulate various degranulation and activation markers such as CD63 and CD203c that can be quantified with flow cytometry in the basophil activation test (BAT) (7, 8). With respect to drugs, it appears that the BAT can be valuable in the diagnostic management of allergy to neuromuscular blocking agents (NMBA) (9-16). However, results of basophil activation experiments with the benzylisoquinoline-derived NMBA atracurium are limited to small series and are generally poorly validated (11-16).

This study aims at assessing the utility of the BAT in atracurium sensitization and to study its contribution in the identification of cross-reactivity between atracurium and other NMBA.

Materials and methods

Study population

As displayed in figure 1, a total of 75 patients, who were referred for diagnostic evaluation after experiencing an hypersensitivity reaction during anaesthesia, were included in the study. Inclusion was based on the clinical history and skin test (ST) results as described in (3). The “+” and “-” in the group names represent a positive or negative skin test to the NMBA used during operation. First, 8 patients who had presented hypotension and/or bronchospasm within 5 minutes after injection of atracurium, demonstrating a positive atracurium ST were included (ATR+). As shown in table 1, 6 patients tested positive in the prick test (SPT), 2 displayed a positive intradermal test (IDT). Second, 7 individuals who experienced anaphylaxis during surgery but did not receive a NMBA and who demonstrated entirely negative ST served as a non-exposed control group (NMBA-). In these patients diagnosis of possible other causes (e.g. latex, chlorhexidine, antibiotics and hypnotics) was established as described elsewhere (3). Third, 29 patients exposed to atracurium who suffered from perioperative anaphylaxis from an alternative cause and demonstrated a negative ST for atracurium (ATR-) were studied.

To investigate the potential of BAT in identifying cross-reactivity between NMBA, 4 additional groups were studied; i) 3 cisatracurium-sensitized patients (CIS+), ii) 9 cisatracurium-exposed patients with negative ST to cisatracurium (CIS-), iii) 14 rocuronium-sensitized patients (ROC+), and finally iv) 5 suxamethonium-sensitized patients (SUX+).

Basophil activation test

The BAT for NMBA is detailed elsewhere (17). Briefly, within 3h after sampling in endotoxin-free heparinised tubes, aliquots of 100 μ L whole blood were stimulated (20 min, 37°C) with buffer containing 2 ng/mL IL-3 (negative control), positive control (anti-IgE 10 μ g/mL, Pharmingen BD Biosciences, Erembodegem, Belgium) or serial dilutions of atracurium 0.5-5,000 μ g/mL. Cells were stained with 2 μ L of a mixture containing anti-CD123-PE, anti-human leukocyte antigen DR-PerCP and anti-CD63-FITC conjugated antibodies (BD, Erembodegem, Belgium). Basophil activation was evaluated by flow cytometric analysis (FACSCalibur, BD, Immunocytometry Systems, San Jose, CA,

USA). Results were expressed as the net percentage of CD63+ basophils, i.e. by subtracting spontaneous CD63 expression (negative control) from the value obtained with allergen (drug) stimulation. A maximum of 5% spontaneous expression of CD63 after stimulation with buffer solution (negative control) was tolerated. The threshold for positivity was calculated by TG-ROC analysis. BAT was consequently considered positive when the optimal dilution of atracurium yielded a positive value. Furthermore, cross reactivity was checked using optimal stimulation concentrations from prior studies (10) or preliminary dose finding experiments.

Total and specific IgE

Total and specific IgE to substituted ammonium compounds (morphine) were quantified with an ImmunoCAP technique (ThermoFisher Scientific, Uppsala, Sweden). Results of morphine sIgE ≥ 0.35 kUA/L were considered positive (18).

Statistics

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 (19).

Results

Atracurium-exposed patients and control individuals (diagnosis)

One ATR+ was non-responder to positive control stimulation and atracurium. She was withdrawn from further analysis but considered false negative for calculation sensitivity and predictive values.

Figure 2A displays the dose-finding experiments between atracurium-sensitized patients (ATR+) and patients not exposed to an NMBA (NMBA-). It emerges ATR+ to show a dose-dependent basophilic appearance of CD63 in response to atracurium. In contrast, expression of CD63 in NMBA- remained merely unchanged and comparable to spontaneous expression of this surface marker. As illustrated by figure 2A dose-finding reveals 500 $\mu\text{g/mL}$ to be the best discriminative stimulation concentration between ATR+ patients and NMBA- individuals, generating a diagnostic threshold value of 5% CD63 up-regulation (fig 2B). For this threshold the BAT was positive in 5/7 ATR+ responders (sensitivity 71%; 95%CI: 29 - 95%) and none of 7 NMBA- (estimated specificity 100%; 95%CI: 59 - 100%). Taking

into account the non-responder; overall sensitivity was 63% (95%CI: 25-91%) and the BAT showed an absolute positive predictive value and a negative predictive value of 70% (95%CI: 35-93%). Note that all except one ATRA+ patients had their BAT experiments within 1 year after the acute event.

The individual BAT results are displayed in table 1 and figure 3. From these it appears that 2 out of 29 ATR- patients test clearly positive in the BAT atracurium. The individual total IgE and sIgE results are summarized in table 1. It appears that 1 ATR+, 2 ATR- and 1 NMBA- patient have a positive sIgE morphine.

Patients exposed to cisatracurium, rocuronium and suxamethonium (assessment of cross-reactivity)

Figure 3 and table 1 summarize the individual percentages of CD63 positive basophils in the patients exposed and/or sensitized to other NMBA. None of the individuals from groups CIS-, ROC+ and SUX+ demonstrated a %CD63 expression exceeding 5% for atracurium. However, BAT atracurium clearly exceeded 5% in 1 out of 3 CIS+ individuals.

In these patients exposed or sensitized to other NMBA a positive sIgE to morphine was found in 1 CIS-, 12 ROC+ and 4 SUX+.

Discussion

Studies about BAT as a diagnostic instrument for rare allergic conditions pose significant challenges and face various difficulties such as accurate identification of sufficient numbers of well-phenotyped patients, identification of optimal agent-specific concentrations and stimulation conditions, establishment and comparison of correct decision thresholds. For the time being, as addressed in the introductory paragraph, the BAT atracurium has been rarely studied, and its methodology and interpretation of results are both topics necessitating attention.

From the current study, which compares the outcome of the BAT atracurium in 8 atracurium sensitized patients to 7 patients not exposed to NMBA, the optimal stimulation concentration was found to be similar to our prior observations with rocuronium (10), enabling the proposal to apply a single protocol and threshold value of 5% for both NMBA. In contrast to former publications (11-15), in our study the decision threshold was calculated by TG-ROC graph analysis and not set arbitrarily. Applying this threshold, and taking into account non-responders, our BAT technique attains a sensitivity of 63%, specificity of 100%, an absolute positive predictive value and a negative predictive

value of 70%. These sensitivity and specificity data are in line with the literature showing sensitivity and specificity of the BAT NMBA to range from 36 to 79% and from 81 to 100% respectively (for review: (8)). A limitation of our calculations is the small number of patients. However, atracurium allergy is a very rare condition, compared with rocuronium or suxamethonium allergy (4). Another limitation could relate to the fact not having included control individuals with uneventful exposure to atracurium. However, such individuals are difficult to enroll for obvious reasons, mainly because they are not referred for diagnostic evaluation. Alternatively, in clinical practice physicians will need to correctly identify the causative compound(s), rather than to dichotomize between patients and asymptomatic control individuals.

Clear positive BAT atracurium responses were found in 2 patients exposed to atracurium but demonstrating a negative ST to this NMBA. Although in the absence of a gold standard it is impossible to draw definite conclusions, in our opinion the high percentage of drug induced basophil responses exceeding 40% suggests an atracurium sensitization overlooked by skin testing, which is currently considered as the reference test to depict NMBA sensitization.

The performance of the BAT atracurium was additionally assessed in various groups of NMBA sensitized or tolerant patients. From this analysis it emerged that BAT atracurium is mirroring absence of ST responsiveness and is negative in patients sensitized to rocuronium and suxamethonium. Thereby indicating that patients who suffer from allergy to aminosteroid-derived NMBA and suxamethonium generally tolerate administration of a benzylisoquinoline (20).

The observation that sIgE towards morphine was positive in the majority of patients sensitized to rocuronium and suxamethonium, but in only one of the 8 atracurium sensitized patients seems to confirm the previously reported lower sensitivity of this morphine-based assays for benzylisoquinoline NMBA compared to its sensitivity for the aminosteroids and suxamethonium (5, 6). In these studies sensitivity of the morphine IgE assay has been reported to vary considerably between 25% (5) and 75% (6) for atracurium, and between 97% (5) and 88.9% (6) for suxamethonium.

Clearly, these findings point to the potential complementarity of BAT to skin testing for NMBA, not only in the diagnosis of atracurium sensitization but also in the study of cross-reactivity between benzylisoquinolines, i.e. atracurium and its cis-isomer cisatracurium. As a matter of fact, as already pointed out elsewhere in the Journal (9, 10), it appears BAT can be useful in patients with high clinical suspicion of NMBA allergy with negative skin tests and can contribute in the identification of safe alternatives for the future. Therefore, because of the unavailability of a gold standard, the potential serious consequences of misdiagnosis, and in line with prior findings for rocuronium (9), our current practice is to recommend BAT atracurium in all patients with a suspicion of

benzylisoquinoline allergy that demonstrate negative or equivocal ST, and to dissuade any further use of atracurium in all patients with a positive BAT, irrespective the ST response to atracurium. However, one should bear in mind that the technique is of no value in non-responders to positive control and allergen stimulation. Alternatively, our data indicate that a morphine-based IgE immunoassay might be unreliable to depict sensitization to benzylisoquinoline NMBA.

Conclusion

In conclusion, the BAT constitutes a useful diagnostic instrument for peri-operative anaphylaxis induced by atracurium. It may serve as a complementary tool that enables quick and simultaneous testing of different potential cross-reactive NMBA and consequently, it is helpful in the search for a safe alternative. In those patients displaying negative or equivocal skin tests, the BAT atracurium will be of great assistance.

Conflict of interest

There are no known conflicts of interest for the authors.

Author's contribution

AU, DGE, JLE, LDC and CHB were involved in study design, data analysis and writing the manuscript. AU, VSO and DGE were involved in patient recruitment.

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Legends of the figures and table

Fig 1 : Description of the 75 patients included in the study. NMBA: neuromuscular blocking agent.

NMBA-: patients with peri-operative anaphylaxis but not exposed to a NMBA. ATR+: patients with atracurium sensitization. ATR-: patients with peri-operative anaphylaxis exposed to atracurium presenting a negative skin test for atracurium. CIS+: patients sensitised to cisatracurium. CIS-: patients with peri-operative anaphylaxis exposed to cisatracurium presenting a negative skin test for cisatracurium. ROC+ patients with rocuronium sensitization. SUX+ patients with suxamethonium sensitization. The "+" and "-" in the group names represent a positive or negative skin test to the NMBA used during operation.

Fig 2A: Mean percentage of CD63+ basophils for different stimulation concentrations of atracurium in atracurium sensitized (ATR+) patients (n = 7, circles) and control individuals whom experienced a peri-operative anaphylaxis, not exposed to NMBA (n = 7, squares). Net percentage was obtained by subtracting the spontaneous CD63 expression from the value obtained with the drug. **Fig 2B:** TG-ROC curve for atracurium-induced CD63 expression generated between ATR+ patients (n = 7) and NMBA- individuals (n=7). Stimulation concentration of atracurium: 500 µg/mL and the optimal threshold of 5% is indicated with the dashed line. Open symbols represent sensitivity whereas closed symbols represent specificity.

Fig 3: Individual percentages of atracurium activated (CD63+) basophils in patients with peri-operative anaphylaxis but not exposed to a NMBA (NMBA-), patients with atracurium sensitization (ATR+), patients with peri-operative anaphylaxis exposed to atracurium presenting a negative skin test for atracurium (ATR-), patients sensitised to cisatracurium (CIS+), patients with peri-operative anaphylaxis exposed to cisatracurium but presenting a negative skin test for cisatracurium (CIS-) patients with rocuronium sensitization (ROC+), patients with suxamethonium sensitization (SUX+). The dashed line indicates the TG-ROC calculated decision threshold for positivity (5%).

Table 1: Characteristics of the 75 patients included in the study. Demographics, Skin test results, sIgE and percentage activated basophils for each patient are given.

NA: not applicable, NAV: not available, ST: skin tests, IDT: Intradermal testing, SPT: skin prick testing, NR: non responder in BAT, M: male, F: female, “-”: negative test *normal value ,**positive if net percentage of CD63 upregulation > 5%,***time between reaction and investigations, expressed in months

NMBA-:patients with peri-operative anaphylaxis but not exposed to a NMBA (NMBA-). ATR+: patients with atracurium sensitization. ATR-: patients with peri-operative anaphylaxis exposed to atracurium presenting a negative skin test for atracurium. CIS+: patients sensitised to cisatracurium. CIS-: patients with peri-operative anaphylaxis exposed to cisatracurium but presenting a negative skin test for cisatracurium. ROC+ patients with rocuronium sensitization. SUX+ patients with suxamethonium sensitization The “+” and “-” in the group names represent a positive or negative skin test to the NMBA used during operation.

PATIENT	SEXE	AGE	IgE tot	slgE morphine ($\leq 0.35\text{ kU/L}$)*	ST SUX	ST VEC	ST ROC	ST ATR	ST CIS	BAT ATR 500**	BAT culprit NMBA	Delay*** (m)
NMBA-	F	67	105	-	-	-	-	-	-	0	NA	10
NMBA-	F	44	268	-	-	-	-	-	-	0	NA	2
NMBA-	F	34	98	-	-	-	-	-	-	0	NA	149
NMBA-	M	67	53	-	-	-	-	-	-	0	NA	2
NMBA-	F	53	26	-	-	-	-	-	-	0	NA	5
NMBA-	F	63	NA	-	-	-	-	-	-	0	NA	2
NMBA-	M	65	268	6	-	-	-	-	-	0	NA	7
ATR+	F	50	178	7.78	-	-	-	SPT1/100	SPT1/10	48	48	2
ATR+	F	63	27	-	-	-	-	SPT1/100	SPT1/1	20	20	2
ATR+	F	43	51	-	-	-	-	SPT1/100	SPT1/100	13	13	3
ATR+	F	50	145	-	-	-	-	SPT1/100	-	16	16	4
ATR+	M	78	28	-	-	-	-	SPT1/10	SPT1/10	9	9	3
ATR+	F	67	2	-	-	IDT1/100	-	IDT1/1000	-	0	0	7
ATR+	F	34	47	-	SPT1/5 IDT1/500	-	-	IDT1/1000	-	0	0	258
ATR+	F	42	271	-	-	-	-	SPT1/100	SPT1/10	NR	NR	7
ATR-	M	48	67	-	-	-	-	-	SPT1/10	45	NA	34
ATR-	F	16	275	-	-	-	-	-	IDT1/100	0	NA	10
ATR-	M	22	900	-	-	-	-	-	-	0	NA	8
ATR-	M	53	741	3.82	-	-	-	-	-	3	NA	1
ATR-	M	46	13	-	-	-	-	-	-	0	NA	3
ATR-	M	72	8550	-	-	-	-	-	-	0	NA	33
ATR-	M	34	56	-	-	-	-	-	-	0	NA	3
ATR-	M	12	288	-	-	-	-	-	-	0	NA	53
ATR-	F	30	23	-	-	IDT1/10	-	-	-	0	NA	2
ATR-	F	52	206	-	-	-	-	-	-	0	NA	12
ATR-	M	19	145	-	-	-	-	-	-	42	NA	1
ATR-	F	42	11	-	-	-	-	-	NAV	0	NA	3
ATR-	F	36	3	-	-	-	-	-	-	0	NA	3
ATR-	F	50	1068	-	-	-	-	-	-	0	NA	134
ATR-	F	29	15	-	-	-	-	-	-	0	NA	12
ATR-	F	19	149	-	-	-	-	-	SPT1/10	0	NA	1
ATR-	F	66	25	-	-	-	-	-	-	0	NA	8
ATR-	M	78	54	-	-	-	-	-	SPT1/10	2	NA	NAV
ATR-	F	17	48	-	-	-	-	-	-	0	NA	16
ATR-	M	50	110	-	-	-	-	-	-	0	NA	1
ATR-	F	36	239	-	-	-	-	-	-	1	NA	4
ATR-	F	38	6	-	-	SPT1/1	-	-	-	0	NA	2
ATR-	F	74	62	-	-	-	-	-	-	1	NA	2
ATR-	F	84	76	1.52	-	-	-	-	-	0	NA	NAV
ATR-	F	55	21	-	-	-	-	-	-	0	NA	NAV

ATR-	M	67	419	-	-	-	-	-	-	0	NA	1
ATR-	F	41	89	-	-	NAV	-	-	-	0	NA	6
ATR-	F	57	86	-	-	IDT1/10	-	-	-	0	NA	2
ATR-	M	58	92	-	-	-	-	-	-	0	NA	4
CIS+	M	46	421	-	-	-	-	SPT1/100	SPT1/10	1	0	36
CIS+	M	54	114	-	-	-	-	-	IDT1/100	0	1	105
CIS+	F	47	27	-	-	IDT1/10	-	-	SPT1/1	68	53	4
CIS-	F	44	8	-	-	-	-	-	-	2	NA	20
CIS-	F	38	451	-	-	-	-	-	-	0	NA	NAV
CIS-	M	83	11	-	-	-	-	-	-	1	NA	NAV
CIS-	F	42	129	-	-	-	-	-	-	0	NA	1
CIS-	M	76	31	-	-	-	-	-	-	0	NA	133
CIS-	F	71	NAV	NAV	-	-	-	-	-	0	NA	29
CIS-	F	29	115	0.55	-	NAV	-	-	-	2	NA	76
CIS-	M	17	210	-	-	NAV	-	-	-	0	NA	NAV
CIS-	M	55	79	-	-	-	-	-	-	1	NA	8
ROC+	F	62	342	1.06	-	-	SPT1/10	-	-	1	85	2
ROC+	M	68	117	0.41	IDT1/1000	-	SPT1/1	-	-	0	9	2
ROC+	F	27	132	8.6	-	NAV	SPT1/10	-	-	0	12	6
ROC+	M	36	40	-	-	-	SPT1/1	-	-	2	89	3
ROC+	F	54	411	16.1	-	SPT1/1 IDT1/100	SPT1/1	-	-	0	26	3
ROC+	M	20	37	-	-	SPT1/1	SPT1/1	-	-	0	0	5
ROC+	F	54	454	17.5	-	-	SPT1/10	-	-	0	0	3
ROC+	F	49	296	22	-	SPT1/1	SPT1/1	-	-	2	15	3
ROC+	F	51	1960	38.2	-	SPT1/1	SPT1/10	-	-	0	36	3
ROC+	F	18	436	6.47	-	-	SPT1/1	-	-	1	65	1
ROC+	F	49	37	0.86	-	SPT1/1	SPT1/1	SPT1/10	-	1	20	30
ROC+	M	60	75	9.79	SPT1/5	SPT1/1	SPT1/10	-	-	0	24	NAV
ROC+	M	48	379	11	SPT1/5	-	SPT1/1	-	-	0	95	11
ROC+	F	46	120	1.46	-	-	SPT1/10	-	-	0	0	13
SUX+	F	48	303	1.52	SPT1/10 SPT 1/5	-	-	-	-	0	0	NAV
SUX+	F	50	325	0.66	SPT1/10	-	-	-	-	2	1	1
SUX+	F	50	311	0.42	SPT1/5	-	-	-	-	0	2	10
SUX+	F	40	485	-	SPT1/10	NAV	-	-	-	1	2	134
SUX+	M	47	484	11.3	SPT1/5	-	-	-	-	0	33	213





