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Skin Reactions to Intradermal Neuromuscular Blocking Agent Injections

A Randomized Multicenter Trial in Healthy Volunteers

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Background: **Numerous reports confirm the performance of intradermal tests for the diagnosis of anaphylaxis during anesthesia; however, there is controversy over their diagnostic value regarding the newer neuromuscular blocking agents (NMBAs).**

Methods: **One hundred eleven healthy volunteers were randomly assigned to receive intradermal injections of two NMBAs, at five increasing concentrations. A concentration was considered as a reactive concentration when it led to a positive reaction in more than 5% of the subjects. These concentrations were compared with the maximal concentration recommended for the diagnosis of sensitization to NMBAs.**

Results: **The maximal nonreactive concentrations were 10^{-3} M for suxamethonium; 10^{-4} M for pancuronium, vecuronium, rocuronium, and cisatracurium; and 10^{-5} M for atracurium and mivacurium. Except for mivacurium, these nonreactive concentrations were close to the maximal concentrations used for the diagnosis of sensitization against NMBAs. For mivacurium, the nonreactive concentrations were higher than the maximal concentration currently recommended in clinical practice.**

Conclusion: **The aminosteroidal NMBAs pancuronium, vecuronium, and rocuronium and the benzylisoquinoline cisatracurium have a similar potency to induce a nonspecific skin reactivity. If the criteria for positivity and the maximal concentrations of the commercially available compounds recommended by French practice guidelines are used, the risk of false-positive results is limited, and only minor modifications of these recommendations could be suggested. A slight reduction in the maximal concentration used for rocuronium from 1:100 to 1:200 and an increase from 1:1,000 to 1:200 for mivacurium can be proposed.**

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DESPITE the large number of reports confirming the performance of prick or intradermal tests for the diagnosis of suspected anaphylactic reaction during anesthesia,[1–22] there is a renewed controversy over their diagnostic value, especially regarding the newer neuromuscular blocking agents (NMBAs).[23–25]

This controversy has been partly supported by the common idea that the incidence of anaphylactic reaction occurring during anesthesia was progressively increasing with time. It was also supported by differences reported regarding their incidence in Europe, Australia, and New Zealand on one hand and in the United States on the other hand.[6,20,26,27] This led some authors to hypothesize that this increase may be partly related to an increased rate of false-positive results when investigating newly commercialized agents as potential offending drugs. However, if we carefully examine results from the most recently published epidemiologic surveys,[20,22,28] things seem to be in sharp contrast. The number of reactions involving NMBAs and latex seems to remain relatively stable, whereas the incidence of anaphylaxis to antibiotics has rapidly increased within the same time period.

Neuromuscular blocking agents are highly charged molecules that have direct vasodilating effects on the skin vasculature.[29] They are able to induce histamine and tryptase release from mast cells within the skin through mechanisms that may vary depending on the compounds studied.[23,29–31] The classic idea that benzylisoquinoline-derived NMBAs have a higher propensity to release histamine from mast cells than the aminosteroidal NMBAs has also been recently contradicted using microdialysis experiments within the skin, which shows that the efficacy of atracurium, but also rapacuronium, an aminosteroidal NMBA, to release histamine was significantly higher than that of other muscle relaxants.[29] Therefore, defining the dose-related wheal-and-flare cutaneous response for each compound is of valuable interest. Indeed, a prospective definition of the histamine-releasing properties of all NMBAs available within the skin in healthy individuals would help us to standardize the diagnostic protocol used by the different investigators, for the diagnosis of sensitization against NMBAs in patients.

In the current study, we investigated all commercially available NMBAs in healthy volunteers. Five increasing log molar concentrations of these NMBAs were injected

intradermally on the forearm and the back to determine a concentration–response relation. These dose–response relations obtained in control subjects were compared with the maximal concentration recommended by the recent French guidelines for the diagnosis of sensitization to NMBAs by intradermal skin tests in subjects who have experienced a suspected anaphylactic reaction during general anesthesia.[32,33]

Subjects and Methods

Subjects

This was a randomized trial in 111 healthy volunteers, performed in two centers specializing in clinical investigation in France (Centre d'Investigation Clinique Inserm–Centre Hospitalier Universitaire de Nancy and Centre d'Investigation Clinique Inserm–Centre Hospitalier Universitaire de Paris Saint Antoine). This study was approved by the Ethical Committee of Paris, Saint Antoine, Paris, France. The subjects were healthy men or nonpregnant, non–breast-feeding women, aged 18–45 yr, who had given proper written informed consent. Exclusion criteria were general anesthesia in the past; atopic diseases such as hay fever, childhood asthma, and atopic dermatitis; a history of hypersensitivity reactions; and known or suspected recent use of steroids, antidepressants, neuroleptics, or antihistamines. Subjects in whom determination of wheals and flares was expected to be difficult because of highly pigmented or suntanned skin were also excluded.

Intradermal Testing

The participants were randomly assigned to receive two NMBAs each. Negative and positive controls and five intradermal injections in increasing concentrations were administered on the forearms and on the back. Each subject received 28 injections, and each NMBA was planned to be administered to 28 subjects. One subject, randomly assigned to vecuronium and cisatracurium, did not present for testing, so in total 27 subjects were tested for these two NMBAs. The intradermal injection was 0.03 ml, giving a wheal of 3–5 mm. All drugs were provided by NV Organon, Boxtel, The Netherlands. The following NMBAs were administered: rocuronium bromide (Org 9426, Esmeron®, NV Organon), rapacuronium bromide (Org 9487, Raplon®; NV Organon), vecuronium bromide (Org NC45, Norcuron®; NV Organon), pancuronium bromide (Org NA97, Pavulon®; NV Organon), atracurium (di)besylate (Tracrium®; GlaxoSmithKline, Marly le Roi, France), cisatracurium (di)besylate (Nimbex®; GlaxoSmithKline), mivacurium (di)chloride (Mivacron®; GlaxoSmithKline), and succinylcholine (di-)chloride (Suxamethonium; Pharmachemie, Haarlem, The Netherlands). The specifics of the available presentations of these NMBAs are presented in table 1.

The NMBAs were used as commercially available and were diluted to molar concentrations of 10^{-7} M (vecuronium, pancuronium, atracurium, cisatracurium, and mivacurium), 10^{-6} M (all), 10^{-5} M (all), 10^{-4} M (all), 10^{-3} M (all), and 10^{-2} M (rocuronium, rapacuronium, and succinylcholine), based on their direct histamine releasing properties. Phenol physiologic diluent (Stallergènes Laboratoires, Antony, France) was used in a 0.4% concentration (4 g phenol in 1,000 ml NaCl, 0.9%) to dilute the NMBAs. It was also used as a negative control. As a second negative control, physiologic saline (NaCl, 0.9%) was used. Histamine (10^{-4} M; Stallergènes Laboratoires) was used in a concentration of 10 μg/ml as a positive control. As a second positive control, codeine (Codeine Phosphate 1%; Stallergènes Laboratoires) was used in a concentration of 50 μg/ml codeine phosphate in phenol physiologic diluent. The assessor was aware of the four control injections administered but was blinded to the NMBAs administered to each subject.

The control solutions were injected first in the following order: saline, phenol physiologic diluent, histamine, and codeine. Independent of the size of the wheals and flares resulting from the injection of these controls, the first NMBA the subject was randomly assigned to receive was then injected on both the forearm and the back, starting with the three lowest concentrations. After confirmation that no systemic reaction had occurred, the remaining two highest concentrations of the NMBA were given. Thereafter, the second NMBA was injected on the forearm and on the back.

Table 1. Product Characteristics

NMBA	Presentation, mg/ml	Molecular Weight	Molar Concentration, M
Rocuronium bromide	10	609.70 (salt)	1.640×10^{-2}
Rapacuronium bromide	20	597.92 (active moiety)	3.345×10^{-2}
Vecuronium bromide	2	637.74 (salt)	3.136×10^{-3}
Pancuronium bromide	2	732.68 (salt)	2.730×10^{-3}
Atracurium (di)besylate	10	1,243.49 (salt)	8.042×10^{-3}
Cisatracurium (di)besylate	2	929.17 (active moiety)	2.152×10^{-3}
Mivacurium (di)chloride	2	1,029.27 (active moiety)	1.943×10^{-3}
Succinylcholine (di)chloride	20	361.31 (salt)	5.536×10^{-2}

NMBA = neuromuscular blocking agent.

Assessments

The diameters of the initial wheals (within 1 min, hereinafter referred to as "the wheal at 1 min") and of the wheals at 15 min were recorded as the mean value of the maximum and perpendicular measured diameter. The reaction to the negative control was considered as normal if there was no increase of the wheal at 15 min as compared with the wheal at 1 min and if there was no flare at 15 min. The reaction to the positive control was considered as normal if there was an increase of the wheal at 15 min as compared with the wheal at 1 min and if a flare was present at 15 min.

The results of the NMBAs were interpreted 15 min after injection as a positive or nonpositive reaction according to the guidelines of the Société Française d'Anesthésie et de Réanimation (SFAR) and the Société Française d'Allergologie et d'Immunologie Clinique (SFAIC).[32] These criteria for positivity are commonly used in the literature and have been proposed by several authors and an expert panel.[5,23,24,34,35] A test result was considered as positive if the mean of the maximum and perpendicular diameter of the wheal at 15 min was at least 8 mm and was at least two times the diameter of the wheal at 1 min. All assessments were performed while the assessor was blinded to the NMBA injected.

Statistical Analysis

Demographic data were summarized by NMBA group. For the positive skin reactions at 15 min, frequency distributions were made for each NMBA, by concentration and location of injection. The size of the wheals at 15 min was summarized for the controls and for each NMBA, by concentration and location of injection. The percentage change in mean wheal diameter at 1 and 15 min after injection was compared statistically for each of the NMBA concentrations. All comparisons were performed with the paired Student *t* test after testing for normality. Statistical testing was two-sided, at a significance level of 0.05. Estimates, with corresponding two-sided 95% confidence intervals, were calculated using the *t* distribution.

Reactive Concentrations

During investigators' meetings, consensus was reached about the concentrations to be considered as "reactive concentrations" able to induce a positive cutaneous response in healthy subjects. Concentrations leading to a positive skin test result (mean of the maximum and perpendicular diameter of the wheal at 15 min was at least 8 mm and was at least two times the diameter of the wheal at 1 min) in more than 5% of the subjects (*i.e.*, 2 subjects or more tested positive per NMBA) were considered as reactive concentrations leading to an unacceptable risk of false-positive results for patients in clinical practice. These concentrations, initially expressed as molar dilutions of each NMBA, were converted into the corresponding ponderal dilution of commercially available compounds. The resulting concentrations considered as able to induce a nonspecific histamine release in healthy subjects were compared with the maximal concentration recommended to be used for intradermal skin testing of patients having presented a suspected anaphylactic reaction during anesthesia recommended in France by the SFAR and the SFAIC.[32]

Results

Demographic Data

The mean age of the volunteers was similar in all NMBA groups (table 2). Male/female distribution did not vary much in the different NMBA groups, except in the groups tested with rocuronium and vecuronium, where the sex distributions were 70%/30% and 30%/70%, respectively.

Skin Reactions

One of the two criteria for determining a reaction as being positive was a wheal size of at least 8 mm 15 min after injection. Results for the different controls and NMBA dilutions are presented separately for the reactions on the forearm and on the back in figures 1 and 2. Additional information regarding this is available on the ANESTHESIOLOGY Web site at http://www.anestheisology-.org. The largest reactions to the highest concentrations were with mivacurium and then rapacuronium, followed by atracurium, cisatracurium, and rocuronium.

The mean wheal at 15 min was statistically significantly larger on the back than on the forearm for both control and NMBA dilutions ($P < 0.01$). At 10^{-4} M, a mean wheal exceeding 8 mm was observed only with mivacurium on both the forearm and the back. A mean wheal exceeding 8 mm was also observed with rapacuronium, but only on the back.

The other criterion that must be fulfilled for determining a reaction as being positive was an increase in wheal

Table 2. Summary of Demographic Data per NMBA

		Age, yr		Sex, n (%)	
NMBA	n	Mean (SD)	Range	Female	Male
Rocuronium	28	28 (7)	21–45	9 (32)	19 (68)
Rapacuronium	28	24 (5)	18–43	11 (39)	17 (61)
Vecuronium	27	27 (7)	18–45	19 (70)	8 (30)
Pancuronium	28	25 (5)	19–39	14 (50)	14 (50)
Atracurium	28	26 (6)	18–45	13 (46)	15 (54)
Cisatracurium	27	26 (4)	18–38	14 (52)	13 (48)
Mivacurium	28	27 (7)	19–45	17 (61)	11 (39)
Succinylcholine	28	26 (5)	20–42	15 (54)	13 (46)

Because per volunteer the skin reaction to two neuromuscular blocking agents (NMBAs) was tested, each volunteer is presented twice in the above table.

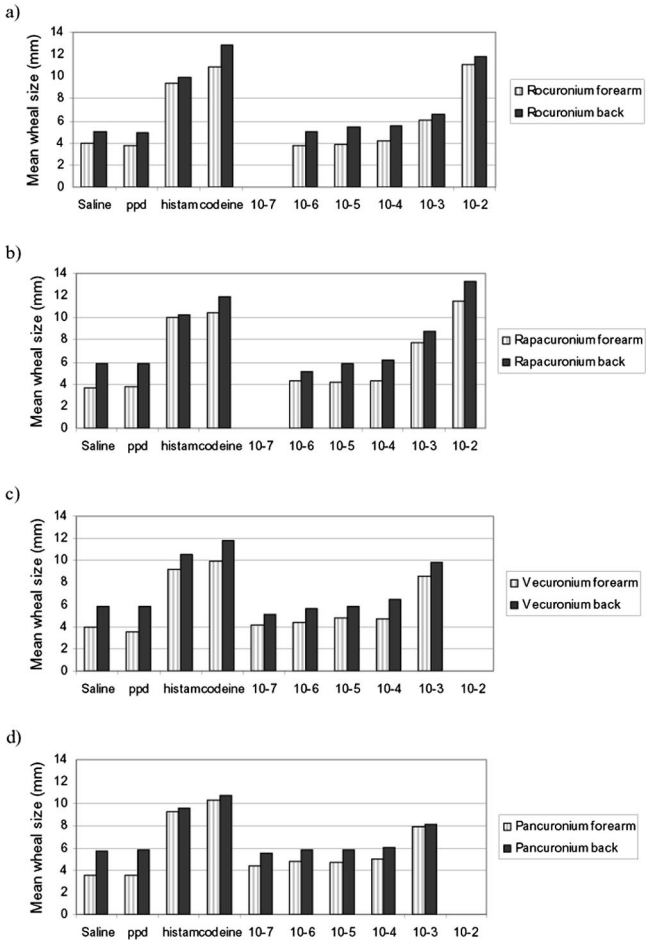

Fig. 1. Wheal size (mean values) at 15 min after intradermal injection of phenol physiologic diluent (ppd; negative control), histamine (histam; positive control), and five increasing log molar aminosteroidal neuromuscular blocking agent concentrations on the forearm or the back in healthy volunteers. (*A*) Rocuronium, (*B*) rapacuronium, (*C*) vecuronium, (*D*) pancuronium.

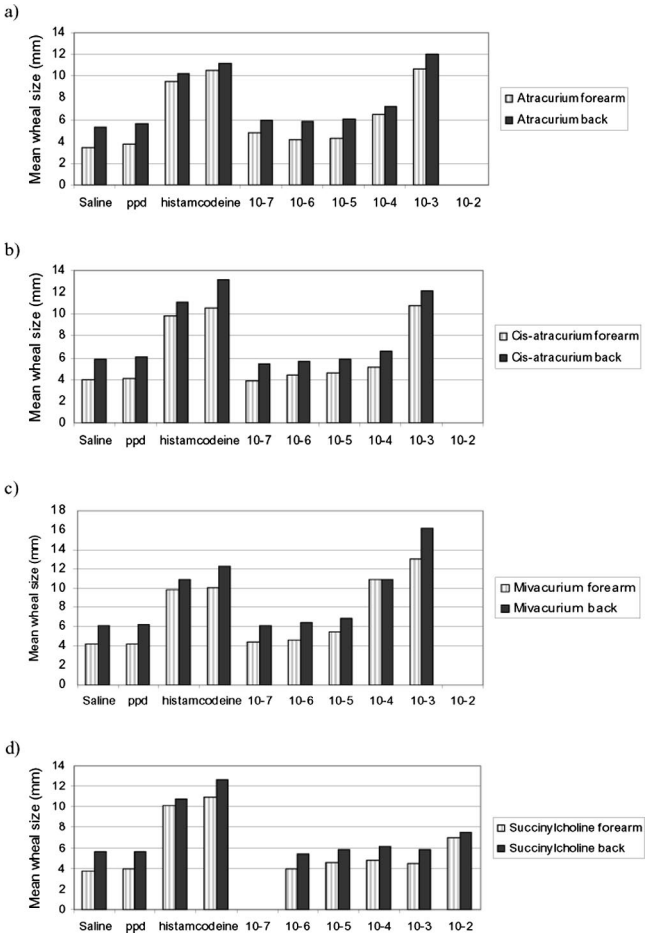

Fig. 2. Wheal size (mean values) at 15 min after intradermal injection of phenol physiologic diluent (ppd; negative control), histamine (histam; positive control), and five increasing log molar benzylisoquinoline neuromuscular blocking agent and succinylcholine concentrations on the forearm or the back in healthy volunteers. (*A*) Atracurium, (*B*) cisatracurium, (*C*) mivacurium, (*D*) succinylcholine.

diameter of at least 100% (*i.e.*, twice the diameter of the initial wheal) at 15 min as compared with 1 min after injection. This is presented in figure 3 for the five different NMBA dilutions, together with the mean percentage change after injection of the negative control (phenol physiologic diluent). The wheal diameter was statistically significantly larger at 15 min for all NMBAs at the highest concentration (10^{-3} log molar dilution of vecuronium, pancuronium, atracurium, cisatracurium, and mivacurium, and 10^{-2} log molar dilution of rocuronium, rapacuronium, and succinylcholine; $P < 0.001$), but the estimated difference was below 100% for vecuronium, pancuronium, and succinylcholine injected on the forearm and for pancuronium and succinylcholine injected on the back. When the next lower concentration (10^{-4} log molar dilution of vecuronium, pancuronium, atracurium, cisatracurium, and mivacurium, and 10^{-3} log molar dilution of rocuronium, rapacuronium, and succinylcholine) was injected, the wheal diameter was statistically significantly larger at 15 min for all NMBAs except for vecuronium, cisatracurium, and succinylcholine injected on the forearm. The estimated difference, however, was more than 100% for mivacurium only.

A skin test was considered as positive when both criteria detailed in the Materials and Methods section were fulfilled. The numbers of patients presenting with a reaction considered as positive for each of the concentrations studied are listed on table 3. In addition, we also reported the NMBA concentrations that induced a statistically significant percentage increase in wheal size at 15 min. To the three lowest NMBA concentrations, an occasional positive reaction (< 5% of cases) was seen either on the forearm or on the back, but not on both, as illustrated in table 3. To the next higher concentration (10^{-4} log molar dilution of vecuronium, pancuronium, atracurium, cisatracurium, and mivacurium, and 10^{-3} log molar dilution of rocuronium, rapacuronium, and succinylcholine), there were more positive reactions, particularly to rapacuronium, atracurium, and mivacurium. To the highest concentrations, there were positive

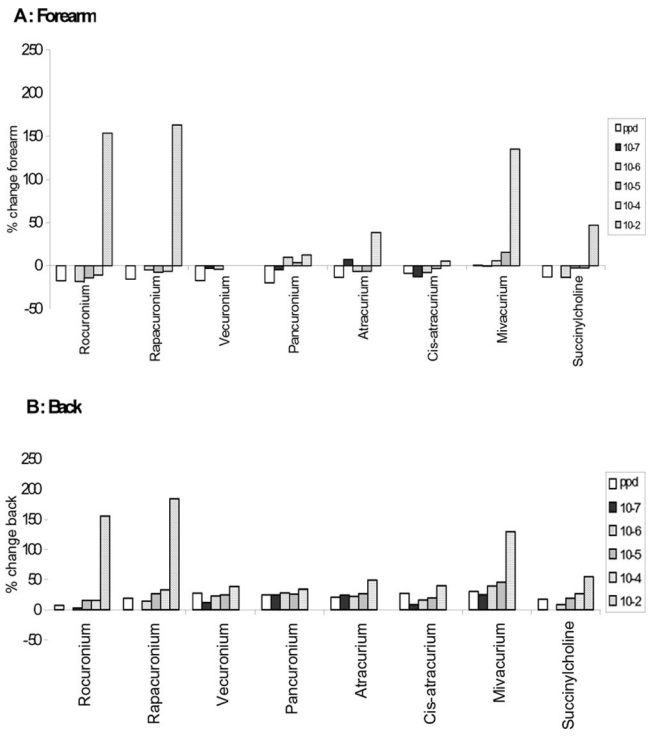

Fig. 3. Percentage change (mean values) in wheal diameters at 1 and 15 min after intradermal injection of phenol physiologic diluent (ppd; negative control) and five increasing log molar neuromuscular blocking agent concentrations on the forearm (*A*) or the back (*B*) in healthy volunteers.

reactions to all NMBAs in more than 5% of the volunteers. At this concentration, positive skin reactions were seen most frequently to rocuronium, rapacuronium, atracurium, cisatracurium, and mivacurium. The results of these tests were similar on the forearm and the back, except for vecuronium, for which no positive reaction was observed at 10^{-4} M on the forearm, whereas two positive reactions were observed on the back.

In addition, table 4 shows the molar dilutions and the corresponding ponderal dilutions of commercially available compounds found to induce a positive skin test result in 5% or more of healthy subjects and the maximal ponderal dilutions recommended to be used for the diagnosis of anaphylaxis in patients having presented an immediate hypersensitivity reaction during anesthesia according to the practice guidelines recommended in France by the SFAR and the SFAIC.

Table 4. Reactive and Nonreactive Concentrations Compared with Current Guidelines

		Healthy Volunteers (n = 120)		
NMBA	mg/ml	Nonreactive Dilution	Reactive Dilution	SFAR Guidelines
Succinylcholine	20	10^{-3} M = 1:55	10^{-2} M = 1:5.5	1:100
Rocuronium	10	10^{-4} M = 1:164	10^{-3} M = 1:16.4	1:100
Vecuronium	2	10^{-4} M = 1:31	10^{-3} M = 1:3.1	1:10
Pancuronium	2	10^{-4} M = 1:27	10^{-3} M = 1:2.7	1:10
Atracurium	10	10^{-5} M = 1:804	10^{-4} M = 1:80.4	1:1,000
Mivacurium	2	10^{-5} M = 1:194	10^{-4} M = 1:19.4	1:1,000
Cisatracurium	2	10^{-4} M = 1:21	10^{-3} M = 1:2.1	1:100

Comparison of nonreactive and reactive concentrations (expressed as molar and corresponding ponderal dilution) of neuromuscular blocking agents (NMBAs) in healthy volunteers with recommended maximal ponderal dilution for the diagnosis of anaphylaxis in patients having presented an immediate hypersensitivity reaction during anesthesia according to guidelines from the French Society of Anesthesiology and Intensive Care (SFAR).

Safety

There were three cases of pruritus after injection of one of the control or test substances. In one subject, this occurred after injection of histamine on the back. Two subjects had pruritus after injection of the highest concentration of atracurium. In one subject, this occurred both on the forearm and on the back. All of these events were mild to moderate and were self-limiting.

Discussion

This is the first study in which all available NMBAs were tested intradermally in five increasing log molar concentrations, in healthy, anesthesia-naive volunteers. The interpretation of the results was based on the criteria recommended by the French Societies of Allergology and of Anesthesiology, which define a positive reaction as a wheal of at least 8 mm in diameter and doubling of the initial wheal, assuming that intradermal injection of

Table 3. Frequency Table of Positive Skin Reactions

		Forearm, n (%)						Back, n (%)					
NMBA	n	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Rocuronium	28		0	0	0	2 (7)*	23 (82)*		0	0*	1 (4)*	2 (7)*	24 (86)*
Rapacuronium	28		0	0	0	6 (21)*	25 (89)*		0	1 (4)*	0*	10 (36)*	26 (93)*
Vecuronium	27	0	0	0	0	8 (30)*		0	0*	0*	2 (7)*	16 (59)*	
Pancuronium	28	0	0	0	1 (4)	10 (36)*		0*	0*	0*	0*	7 (25)*	
Atracurium	28	1 (4)	0	0	4 (14)*	25 (89)*		0*	1 (4)*	0*	2 (7)*	25 (89)*	
Cisatracurium	27	0	0	0	0	24 (89)*		0	1 (4)*	0*	0*	23 (85)*	
Mivacurium	28	0	0	1 (4)*	20 (71)*	28 (100)*		0*	0*	0*	23 (82)*	26 (93)*	
Succinylcholine	28		0	0	1 (4)	0	4 (14)*		0	0*	0*	0*	3 (11)*

Because per volunteer the skin reaction to two neuromuscular blocking agents (NMBAs) was tested, each volunteer is presented twice in the above table.

* NMBA concentrations inducing a statistically significant percentage increase in wheal size at 15 min as compared with 1 min.

0.03 ml causes an initial wheal of 4 mm.[32] These criteria have been defined according to the currently available literature and have been proposed by several authors and an expert panel.[5,23,24,34,35]

Skin tests for immune-mediated hypersensitivity reactions are based on the observation of the local consequences of histamine release by sensitized cutaneous mast cells induced by the allergen. Cell activation results from bridging of specific immunoglobulin E (IgE) by at least divalent allergens. This could explain why skin tests for the diagnosis of IgE-dependent allergies to protein allergens, which usually contain numerous epitopes, are considered as more efficient than for the diagnosis of allergy to drugs whose epitopes are usually unknown and could be only monovalent. However, NMBAs carry two quaternary ammonium ions that are considered as the main epitopes involved in anaphylaxis to these drugs.[36] This is probably why the performance of skin tests for the diagnosis of sensitization against NMBAs has been considered as excellent by most of the authors.[1,2,5,7,9,11–13,15,17,18,21,37,38] Indeed, the diagnostic value of an intradermal test in patients having presented with a suspected IgE-mediated immediate hypersensitivity reaction during anesthesia was established many years ago using Prausnitz-Kustner testing in human subjects.[11,37,39] Its excellent reproducibility[10] as well as persistence of positivity with time[10,40,41] has also been confirmed. Therefore, when a neuromuscular blocking drug is incriminated as the cause of a reaction, it has been shown that the negativity of intradermal tests to other NMBAs allows for a subsequent safe use of these negative drugs.[42] Nevertheless, no diagnostic investigation can be considered as 100% reliable, and some cases of false-negative results of intradermal tests leading to reexposure of patient to these NMBAs and renewed adverse reactions have been reported.[43,44] However, these authors used a 10^{-3} dilution of the commercially available NMBAs to increase the specificity of their diagnostic procedure.

Intradermal tests to NMBAs can be performed either on the anterior aspect of the forearm or on the back, and there is no consensus at this time. Nevertheless, mast cell distribution varies significantly between trunk and distal body sites,[45,46] and skin sensory innervation is not uniform.[47] Some authors favor intradermal tests on the back because the skin is less exposed to and modified by physical agents.[13] However, in a report regarding a prick test, the skin reactivity was less notable on the forearm than on the back for both histamine and allergen.[48] At that time, such data were lacking for intradermal tests and NMBAs. In our series, the overall results of intradermal tests were similar on the forearm and the back, except for vecuronium, for which no positive reaction was observed to 10^{-4} on the forearm, whereas two positive reactions were observed on the back. However, the mean wheal at 15 min was statistically significantly larger on the back than on the forearm for both control and NMBA dilutions.

In this study, interpretation was based on the SFAR criteria, which define a positive reaction as a wheal of at least 8 mm in diameter and doubling of the initial wheal, assuming that intradermal injection of 0.03 ml causes an initial wheal of 4 mm.[32] Indeed, intradermal tests consists of an injection, at a 0.5- to 1-mm depth, of a volume between 0.02 and 0.05 ml to obtain an injection papula of 3–5 mm.[13,15] However, in routine practice, it is difficult to fix a strict volume using an intradermal syringe. In our study, however, we found an initial wheal of 5 mm in most cases. This clearly emphasizes the importance of taking into account not only the final wheal diameter obtained 15 min after the injection, but also the ratio between this wheal and the initial papula diameter obtained at the time of injection to interpret intradermal test results. The measurement of the initial wheal and the requirement that the wheal is doubled are essential and should not be disregarded. Failure to fulfill these requirements would result in diminished specificity and hence a higher level of false-positive results.

One of the limitations of intradermal tests for the diagnosis of sensitization to NMBAs comes from their ability to induce histamine release from mast cells within the skin through non–IgE-mediated mechanisms.[23,29–31,49,50] Thus, false-positive results may occur, depending on the maximal concentration used, but this concentration will vary from one compound to another, according to their respective nonspecific histamine-releasing properties. This difficulty has long been recognized in reports that demonstrated the limited predictive value of skin tests used as screening tests.[4,51–53]

Two studies have been reported in which the skin reactions to log molar concentrations of intradermally injected NMBAs were evaluated in healthy volunteers, but these series only investigated cisatracurium and rocuronium.[23,24] Our series is the only one investigating all NMBAs commercially available. Although positivity criteria in previous studies differ from the SFAR criteria, in all of these three studies, increasing NMBA concentrations resulted in increasing wheal diameters. In previous reports, depending of the criteria used, positivity was observed at a 10^{-3} log molar concentration for rocuronium and at a 10^{-4} log molar concentration for cisatracurium.[23] In our series, using the more restrictive SFAR criteria, a significant number of positive reactions ($> 5\%$ of reacting subjects) were observed only at a 10^{-3} log molar concentration for both rocuronium and cisatracurium. Therefore, histamine releasing properties within the skin seem to be similar for the most recent NMBAs (table 4).

Prick tests, which are performed by perforating the skin approximately 1 mm deep, through a drop of test solution with a needle or a special device, are also largely used to investigate skin reactivity to NMBAs.[5,9,12,16]

However, these tests have been considered as less sensitive than intradermal tests by some authors.[15] Their specificity has recently been questioned in a study investigating skin sensitivity to rocuronium and vecuronium in healthy volunteers.[25] On the contrary, in another series investigating cisatracurium and rocuronium, no positive response was observed even with undiluted solution in healthy volunteers.[24] Systematic investigation of the maximal reactive and nonreactive concentrations for all NMBAs in healthy volunteers would be interesting.

It is of special interest to establish a dose-related skin reaction curve in healthy subjects for all NMBAs used in standard clinical practice. However, one should be aware of the different conclusions that should be drawn from results obtained from healthy subjects, when designing a diagnosis procedure that will be used for patients. Indeed, the predictive value of a test depends not only on the performance of the test itself but also on the prevalence of the disease in the population tested.[54]

If the test is used to screen patients for anesthetic allergy in the absence of any abnormal reaction, the prevalence of the disease is low and the predictive value of the test will be minimized. In such situations, a false-positive rate of 5% will be unacceptable.[54] Therefore, skin tests are not recommended before anesthesia in apparently healthy subjects without any specific risk factors.[32]

The situation for patients having presented a suspected immediate hypersensitivity reaction during anesthesia is completely different. In this population, the prevalence of the disease is high and the predictive value is maximized. That is why, in our series, a rate of less than 5% positive reaction in healthy subjects has been considered as an acceptable threshold to determine the maximal concentrations that should be used for the diagnosis of anaphylaxis in patients having presented an immediate hypersensitivity reaction during anesthesia.

One should also notice that there is no accepted standard diagnostic method, such as a challenge test, for the diagnosis of sensitization to NMBAs. Therefore, our aim will be to obtain the highest possible degree of clinical safety for each individual who is referred to an allergo-anesthesia consultation. Therefore, a diagnosis of IgE-mediated immediate hypersensitivity will not be determined by a single test criterion, but by a weight of evidence that includes the description of the reaction and test results.

We can compare both reactive and nonreactive concentrations obtained in healthy subjects to the maximal concentration recommended in France for patients with a history of suspected anaphylaxis during anesthesia (table 4). In standard clinical practice, allergologists do not use molar dilution of NMBAs but a 10-fold dilution of the commercially available concentration of the drugs. Therefore, log molar concentrations have been converted into the corresponding 10-fold dilutions of commercial compounds.

It seems that the maximal dilutions recommended in the SFAR and SFAIC practice guidelines are close to the maximal concentrations considered as negative in healthy volunteers (suxamethonium 1:100 *vs.* 1:55, rocuronium 1:100 *vs.* 1:164, vecuronium 1:10 *vs.* 1:31, pancuronium 1:10 *vs.* 1:27, atracurium 1:1,000 *vs.* 1:804) and clearly below the concentrations considered as positive (table 4). However, concentrations recommended for aminosteroidal compounds are slightly higher than those showed as negative in healthy volunteers, whereas the reverse is true for benzylisoquinoline compounds. Therefore, if one wishes to obtain a similar picture for both compound families, a slight modification in the recommendation could be proposed, leading to the following maximal concentrations: rocuronium 1:200 (instead of 1:100), vecuronium 1:50 (instead of 1:10), pancuronium 1:50 (instead of 1:10), and mivacurium 1:200 (instead of 1:1,000). However, one should keep in mind that the current study refers to the histamine-releasing properties of NMBAs within the skin in healthy subjects, not the diagnostic predictive value in patients with a history of immediate hypersensitivity reaction during anesthesia. The risk of increased false-negative results when using overdiluted concentrations does exist,[43] and the maximal concentrations used to perform intradermal tests must represent the best compromise between sensitivity and specificity of the diagnostic procedure.

Conclusion

All NMBAs are able to induce a nonspecific cutaneous wheal response, depending on the concentration used. Therefore, determination of the maximal nonreactive concentration for each drug within healthy subjects seems to be of importance to determine the maximal concentrations that should be used for the diagnosis of anaphylaxis. Our results show that the various commercially available aminosteroidal NMBAs pancuronium, vecuronium, and rocuronium and the benzylisoquinoline cisatracurium have a similar potency in producing positive cutaneous responses. If the criteria for positivity and the maximal concentrations recommended by current practice guidelines in France are used, the risk of false-positive results is limited, and only minor modifications of these recommendations should be proposed.

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References

1. Fisher MM: Intradermal testing after severe histamine reactions to intravenous drugs used in anaesthesia. Anaesth Intensive Care 1976; 4:97–104

2. Fisher MM: Intradermal testing in the diagnosis of acute anaphylaxis during anaesthesia: Results of five years experience. Anaesth Intensive Care 1979; 7:58–61
3. Fisher MM: The diagnosis of acute anaphylactoid reactions to anaesthetic drugs. Anaesth Intensive Care 1981; 9:235–41
4. Fisher MM: Skin testing in the preoperative diagnosis of anaesthetic allergy. Ann Fr Anesth Reanim 1985; 4:192–4
5. Fisher MM, Bowey CJ: Intradermal compared with prick testing in the diagnosis of anaesthetic allergy. Br J Anaesth 1997; 79:59–63
6. Fisher M: Anaphylaxis to anaesthetic drugs. Novartis Found Symp 2004; 257:193–202
7. Galletly DC, Treuren BC: Anaphylactoid reactions during anaesthesia: Seven years' experience of intradermal testing. Anaesthesia 1985; 40:329–33
8. Laxenaire MC, Moneret-Vautrin DA, Boileau S, Moeller R: Adverse reactions to intravenous agents in anaesthesia in France. Klin Wochenschr 1982; 60: 1006–9
9. Leynadier F, Sansarricq M, Didier JM, Dry J: Prick tests in the diagnosis of anaphylaxis to general anaesthetics. Br J Anaesth 1987; 59:683–9
10. Leynadier F, Sansarricq M, Dry J: Reproducibility of intradermal tests after anaphylaxis caused by muscle relaxants. Presse Med 1987; 16:523–5
11. Moneret-Vautrin DA, Laxenaire MC, Moeller R: Anaphylaxis due to succinylcholine: Immunoallergological study of thirteen cases. Clin Allergy 1981; 11:175–83
12. Moneret-Vautrin DA, Laxenaire MC, Widmer S, Hummer M: The value of prick tests in the detection of anaphylaxis caused by muscle relaxants. Ann Fr Anesth Reanim 1987; 6:352–5
13. Moneret-Vautrin DA, Laxenaire MC: Skin tests in diagnosis of allergy to muscle relaxants and other anesthetic drugs. Monogr Allergy 1992; 30:145–55
14. Moneret-Vautrin DA, Kanny G, Gueant JL, Widmer S, Laxenaire MC: Prevention by monovalent haptens of IgE-dependent leucocyte histamine release to muscle relaxants. Int Arch Allergy Immunol 1995; 107:172–5
15. Moneret-Vautrin DA, Kanny G: Anaphylaxis to muscle relaxants: rational for skin tests. Allerg Immunol (Paris) 2002; 34:233–40
16. Pepys J, Pepys EO, Baldo BA, Whitwam JG: Anaphylactic/anaphylactoid reactions to anaesthetic and associated agents: Skin prick tests in aetiological diagnosis. Anaesthesia 1994; 49:470–5
17. Sage D: Intradermal drug testing following anaphylactoid reactions during anaesthesia. Anaesth Intensive Care 1981; 9:381–6
18. Vervloet D, Arnaud A, Vellieux P, Kaplanski S, Charpin J: Anaphylactic reactions to muscle relaxants under general anesthesia. J Allergy Clin Immunol 1979; 63:348–53
19. Vervloet D, Nizankowska E, Arnaud A, Senft M, Alazia M, Charpin J: Adverse reactions to suxamethonium and other muscle relaxants under general anesthesia. J Allergy Clin Immunol 1983; 71:552–9
20. Mertes PM, Laxenaire MC, Alla F: Anaphylactic and anaphylactoid reactions occurring during anesthesia in France in 1999–2000. ANESTHESIOLOGY 2003; 99:536–45
21. Mertes PM, Laxenaire MC: Adverse reactions to neuromuscular blocking agents. Curr Allergy Asthma Rep 2004; 4:7–16
22. Mertes PM, Laxenaire MC: Anaphylactic and anaphylactoid reactions occurring during anaesthesia in France: Seventh epidemiologic survey (January 2001-December 2002). Ann Fr Anesth Reanim 2004; 23:1133–43
23. Levy JH, Gottge M, Szlam F, Zaffer R, McCall C: Weal and flare responses to intradermal rocuronium and cisatracurium in humans. Br J Anaesth 2000; 85:844–9
24. Berg CM, Heier T, Wilhelmsen V, Florvaag E: Rocuronium and cisatracurium-positive skin tests in non-allergic volunteers: Determination of drug concentration thresholds using a dilution titration technique. Acta Anaesthesiol Scand 2003; 47:576–82
25. Dhonneur G, Combes X, Chassard D, Merle JC: Skin sensitivity to rocuronium and vecuronium: A randomized controlled prick-testing study in healthy volunteers. Anesth Analg 2004; 98:986–9
26. Bhananker SM, O'Donnell JT, Salemi JR, Bishop MJ: The risk of anaphylactic reactions to rocuronium in the United States is comparable to that of vecuronium: An analysis of food and drug administration reporting of adverse events. Anesth Analg 2005; 101:819–22
27. Harboe T, Guttormsen AB, Irgens A, Dybendal T, Florvaag E: Anaphylaxis during anesthesia in Norway: A 6-year single-center follow-up study. ANESTHESIOLOGY 2005; 102:897–903
28. Laxenaire MC, Mertes PM: Anaphylaxis during anaesthesia: Results of a two-year survey in France. Br J Anaesth 2001; 87:549–58
29. Koppert W, Blunk JA, Petersen LJ, Skov P, Rentsch K, Schmelz M: Different patterns of mast cell activation by muscle relaxants in human skin. ANESTHESIOLOGY 2001; 95:659–67
30. Galletly DC: Comparative cutaneous histamine release by neuromuscular blocking agents. Anaesth Intensive Care 1986; 14:365–9
31. Levy JH, Adelson D, Walker B: Wheal and flare responses to muscle relaxants in humans. Agents Actions 1991; 34:302–8
32. Reducing the risk of anaphylaxis during anaesthesia. Abbreviated text. Ann Fr Anesth Reanim 2002; 21:7s–23s
33. Mertes PM, Laxenaire MC, Lienhart A, Aberer W, Ring J, Pichler WJ, Demoly P: Reducing the risk of anaphylaxis during anaesthesia: Guidelines for clinical practice. J Investig Allergol Clin Immunol 2005; 15:91–101
34. Mertes PM, Laxenaire MC: Allergic reactions occurring during anaesthesia. Eur J Anaesthesiol 2002; 19:240–62
35. Van Metre TE Jr, Adkinson NF Jr, Kagey-Sobotka A, Marsh DG, Normal PS, Rosenberg GL: How should we use skin testing to quantify IgE sensitivity? J Allergy Clin Immunol 1990; 86:583–6
36. Baldo BA, Fisher MM: Substituted ammonium ions as allergenic determinants in drug allergy. Nature 1983; 306:262–4
37. Vervloet D, Arnaud A, Vellieux P, Kaplanski S, Charpin J: Anaphylactic type of accidents due to myorelaxing agents during general anesthesia: Clinical and biological study. Nouv Presse Med 1977; 6:725–8
38. Vervloet D: Allergy to muscle relaxants and related compounds. Clin Allergy 1985; 15:501–8
39. Fisher MM: Reaginic antibodies to drugs used in anesthesia. ANESTHESIOLOGY 1980; 52:318–20
40. Fisher MM, Baldo BA: Persistence of allergy to anaesthetic drugs. Anaesth Intensive Care 1992; 20:143–6
41. Didier A, Benzarti M, Senft M, Charpin D, Lagier F, Charpin J, Vervloet D: Allergy to suxamethonium: Persisting abnormalities in skin tests, specific IgE antibodies and leucocyte histamine release. Clin Allergy 1987; 17:385–92
42. Leynadier F, Calinaux C, Dry J: Predictive value of intradermal tests using muscle-relaxing drugs. Ann Fr Anesth Reanim 1989; 8:98–101
43. Fisher MM, Merefield D, Baldo B: Failure to prevent an anaphylactic reaction to a second neuromuscular blocking drug during anaesthesia. Br J Anaesth 1999; 82:770–3
44. Fraser BA, Smart JA: Anaphylaxis to cisatracurium following negative skin testing. Anaesth Intensive Care 2005; 33:816–9
45. Janssens AS, Heide R, den Hollander JC, Mulder PG, Tank B, Oranje AP: Mast cell distribution in normal adult skin. J Clin Pathol 2005; 58:285–9
46. Weber A, Knop J, Maurer M: Pattern analysis of human cutaneous mast cell populations by total body surface mapping. Br J Dermatol 2003; 148:224–8
47. McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW: Epidermal nerve fiber density: normative reference range and diagnostic efficiency. Arch Neurol 1998; 55:1513–20
48. Nelson HS, Knoetzer J, Bucher B: Effect of distance between sites and region of the body on results of skin prick tests. J Allergy Clin Immunol 1996; 97:596–601
49. Galletly DC, Treuren BC: Cutaneous sensitivity to atracurium and vecuronium in patients suffering anaphylactoid reactions to neuromuscular blockers. Anaesth Intensive Care 1985; 13:305–10
50. Lavery GG, Clarke RS, Watkins J: Histaminoid response after intradermal and intravenous administration of atracurium, vecuronium and tubocurarine: A comparative study. Eur J Anaesthesiol 1986; 3:439–47
51. Maria Y, Grosdidier R, Haberer JP, Moneret-Vautrin DA: Prospective preoperative survey of 300 patients using prick tests with muscle relaxants. Ann Fr Anesth Reanim 1989; 8:301–5
52. Moneret-Vautrin DA, Laxenaire MC: Predictive checkup for risk of anaphylactoid shock in anesthesia. Monogr Allergy 1992; 30:156–61
53. Porri F, Pradal M, Rud C, Charpin D, Alazia M, Gouin F, Vervloet D: Is systematic preoperative screening for muscle relaxant and latex allergy advisable? Allergy 1995; 50:374–7
54. Fisher MM, Doig GS: Prevention of anaphylactic reactions to anaesthetic drugs. Drug Saf 2004; 27:393–410