

MUSCLE RELAXANTS ALLERGY

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The most common agents that are responsible for intraoperative anaphylaxis are muscle relaxants. In fact, neuromuscular blocking agents (NMBAs) contribute to 50-70% of allergic reactions during anaesthesia. The main mechanism of hypersensitivity reactions to NMBAs is represented by acute type I allergic reactions and the most severe form is anaphylaxis. The rate of non IgE mediated immediate hypersensitivity reactions usually varies between 20 % and 35% of the reported cases in most large series. In a recent report, non allergic suspected reactions to NMBAs occurred with almost the same frequency as did those with an allergic component. Although the precise mechanisms of these reactions remain difficult to ascertain, they usually result from direct non specific mast cell and basophil activation. After diagnostic procedures, regardless of the specific IgE results, NMBAs are contraindicated if the skin tests were positive. In view of the constantly evolving anesthesiologic practices, and of the complexity of allergy investigation, an active policy to identify patients at risk and to provide any necessary support to anaesthetists and allergologists should be promoted. The high frequency of IgE anaphylactic reactions and the feasibility of skin tests in children justify systematic allergy testing whenever hypersensitivity reaction occurs during general anaesthesia.

Neuromuscular blocking agents (NMBAs) are the leading drugs responsible for immediate hypersensitivity reactions during anaesthesia. Adequate muscle relaxation is a sine qua non of many surgical procedures: e.g. to prevent patient movement and injury, to permit surgical access to body cavities, to prevent shivering and decrease oxygen consumption. It is an important component of anaesthetic technique per se facilitating endotracheal intubation, mechanical ventilation of the lungs and the treatment of laryngeal spasm (1).

NMBAs are quaternary ammonium compounds structurally related to acetylcholine (ACh). They impede impulse transmission at the neuromuscular junction binding to the subunit of the ACh receptor. There are structural differences between depolarizing muscle relaxants (leptocurares) which are thin, flexible molecules, and nondepolarizing muscle relaxants

(pachycurares), which are large, rigid ring systems into which the quaternary groups are incorporated (2).

Depolarizing (non competitive) NMBAs bind to the α subunit of the ACh receptor thus initiating depolarization of the membrane and muscle contraction. NMBAs are not rapidly hydrolyzed by acetyl cholinesterase but, leaving the ion channel in a depolarized state, render it refractory to further ACh, i.e. giving a depolarizing or non competitive block. There are no antagonists to depolarizing NMBAs such as suxamethonium, succinylcholine, and decamethonium.

Suxamethonium, a dimer of ACh, has a very fast onset but short duration of action, that make it an indispensable tool in the management of anaesthetic emergency situations, when rapid control of the airway is required (e.g. "crash" induction, laryngospasm) (1).

Succinylcholine has a rapid onset (30-60 sec.) and a

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short duration of action (3–5 min.). It mimics the action of Ach at the neuromuscular junction by depolarizing the postjunctional membrane, provided that the depolarization is sustained until succinylcholine is hydrolyzed.

Decamethonium is a short acting depolarizing muscle relaxant and is similar to Ach. It acts as a partial agonist of the nicotinic Ach receptor at the motor endplate.

Non depolarizing NMBAs are either curare alkaloids, benzyliisoquinolinium or aminosteroid compounds. In clinical practice, the choice of non-depolarizer is based on side effects, route of elimination and most importantly duration of action. Available nondepolarizing relaxants can be classified according to chemical class: the steroidal compounds (pancuronium, vecuronium, rocuronium) and the benzyliisoquinolinium substances (atracurium, cisatracurium, mivacurium). Pancuronium does not cause histamine release or ganglionic blockade; it does cause a vagolytic tachycardia. It is also long-acting and is excreted by kidneys. Vecuronium, an intermediate acting aminosteroid causes neither ganglionic blockade, histamine release nor tachycardia and is metabolized by the liver. Rocuronium is similar but causes a slight tachycardia. It has been used in high doses to provide a very rapid onset but has an intermediate duration of action. Atracurium causes some histamine release and hypotension, which is offset by a compensatory increase in heart rate and is broken down by non organ enzyme Hoffman degradation. Cisatracurium is the R-cis isomer of atracurium. Cisatracurium and atracurium share a similar pharmacokinetics profile, suggesting that equivalent doses of these agents should have a similar duration of action (1).

However, in children, the potency of cisatracurium is approximately six times that of atracurium. Mivacurium is the shortest acting non-depolariser agent currently available, even though it is not so short-acting to replace suxamethonium (1).

EPIDEMIOLOGY

The most common agents that are responsible for intraoperative anaphylaxis are muscle relaxants, in fact, NMBAs contribute to 50-70% of allergic reactions during anaesthesia (3, 4).

The exact prevalence of anaphylaxis during anaesthesia is difficult to establish (5). Possible bias include uncertainties over reporting accuracy and exhaustiveness, differences in anaesthetic practices between centers or countries. The quality of information obtained from multiple cases under circumstances will necessarily be variable regarding completeness and precision, being detailed case history an important part of the diagnosis (6). In addition, the surveillance and analysis of adverse

drug reactions is difficult because these reactions are rare, random and mostly independent of repeated exposure of patients at low risk (7).

Within these limits, the estimated incidence of all immune and non-immune-mediated hypersensitivity reactions during anaesthesia varied from 1 in 5000 to 1 in 13000 anaesthetics in Australia, 1 in 5000 in Thailand, 1 in 1250 to 1 in 5000 in New Zealand, 1 in 3500 in England, 1 in 3000 to 1 in 11000 in Norway, 1 in 6500 in France (8). In Italy, there have been no studies regarding the incidence of allergic reactions during anaesthesia.

NMBAs represent around 58.2% of anaphylactic reactions in France (3), 93.2% in Norway (9), 37% in Spain (10), 69% in UK (6). On the contrary, IgE mediated reactions involving NMBAs appear to be far less frequent in Denmark and Sweden (9) and allergic reactions to NMBAs have also been reported for smaller series in the United States (11).

Although rare, IgE-mediated hypersensitivity reactions may lead to death, even when appropriately treated, with mortality ranging from 3.5% in Australia, 4.7% in Japan to 10% in United Kingdom (12). It should also be noted that in 15% to 50% of cases, anaphylaxis to NMBAs is reported in absence of any previous exposure to the drug (8).

A recent report (10) analyzed adverse drug reactions to seven NMBAs (tubocurarine, suxamethonium, alcuronium, atracurium, pancuronium, vecuronium, mivacurium) over a 30-year period of time. Out of 950 adverse drug reactions reported, 9% had a fatal outcome. In some reports (3, 9) suxamethonium appeared to be more frequently involved, with some differences reflecting variations in anaesthetic practices between one country to another. In contrast, pancuronium and cis-atracurium are associated with the lowest incidence of anaesthetic anaphylaxis in large series (3).

A trend concerning an increased frequency of allergic reactions to rocuronium was initially reported in Norway and France (13), but not in Australia (14) and United Kingdom. Some authors suggest that the incidence of anaphylactic reactions to NMBAs merely reflects their market share (clinical use) (14, 15). It remains uncertain whether these reports represent the experience of the larger anaesthesiology community. Further large epidemiologic studies will be necessary to elucidate this problem.

There are very few studies about incidence of anaphylaxis during anaesthesia in children. In a recent report, IgE mediated anaphylaxis during anaesthesia was diagnosed in 51 children in a French paediatric population over a 12 year period. The NMBAs were responsible for 60.8% (31 out of 51) of cases of IgE mediated anaphylaxis. Adverse reactions to NMBAs appeared to become more frequent with age, although no significant association was

Table 1. Level of severity for quantification of immediate hypersensitivity reactions

Level	Signs and symptoms
I	Cutaneous signs: generalized erythema, urticaria, angioedema
II	Measurable but not life-threatening symptoms Cutaneous signs, hypotension, tachycardia Respiratory disturbance: cough, difficulty to inflate
III	Life threatening symptoms: collapse, tachycardia or bradycardia, arrhythmias, bronchospasm
IV	Cardiac and/or respiratory arrest

Modified from: Mertes P et al. Hypersensitivity reactions to Neuromuscular blocking agents. *Current Pharmaceutical Design* 2008;14: 2809-25.

Table 2. Differential diagnosis of anaesthesia-related anaphylaxis

Symptoms and signs	Cause
Skin and mucosa (hives, flush, erythema, urticaria) swelling head and neck	Direct histamine release Venous obstruction Head down position C1-esterase inhibitor deficiency Mastocytosis
Airways comprise, dyspnoea, wheeze, bronchospasm, stridor, difficulty in inflating the lungs	Direct histamine release Acid aspiration Exacerbation of asthma Intubation Oesophageal or bronchial intubation Difficulty airways
Fall in blood pressure	Direct histamine release Visceral traction Vasodilatation by drugs Cardiac drug effects Drug overdose and interactions Gas embolism Vaso-vagal reaction Electrolyte disorders Hypoxemia Concealed hypovolemia

Modified from: Ebo DG et al. Anaphylaxis during anesthesia: diagnostic approach. *Allergy* 2007;62:471-487.

found. No significant relationship was found between the type of drug, and the character of IgE mediated or not IgE mediated reaction and the severity. No significant relationship was found between the occurrence of anaphylactic reaction during anaesthesia and the number of previous aesthetic procedures (16).

MECHANISMS

IgE-mediated hypersensitivity reactions

The main mechanism of hypersensitivity reactions to

NMBAs is represented by acute type I allergic reactions and the most severe form is anaphylaxis. NMBAs are low molecular weight molecules and have been usually considered as haptens incapable of inducing the production of drug-specific antibodies by themselves. Consequently, prior conjugation of the drug or of one of its degradation products with a protein carrier is usually regarded as the initial step of sensitization. The following processing by professional antigen-presenting cells before presentation of peptides on the cell surface is the second step in close association with a class I or II histocompatibility molecule (1).

In this way, NMBAs can elicit IgE dependent release of potent, pharmacologically active preformed mediators such as histamine and tryptase from mast cells and basophils.

The main antigenic determinants involved in the generation of specific IgE antibodies are substituted ammonium ions, as demonstrated by Baldo (1). It has been hypothesized that most NMBAs, which bear two similar quaternary ammonium ions per molecule, are capable of bridging IgE antibodies and eliciting anaphylaxis. Therefore, the flexibility of the chain between the ammonium ions as well as the distance between the quaternary ammonium ions might be important during the elicitation phase of the anaphylaxis (1). Flexible molecules, such as succinylcholine, can stimulate sensitized cells more strongly than rigid molecules (i.e. pancuronium). If this would be further confirmed by epidemiologic surveys, propenyl ammonium groups, present in both NMBAs, might be involved in this apparent increased allergenicity (1).

Because of the presence of substituted and ubiquitous ammonium ions in each of the commonly used NMBAs, allergic sensitivity to one NMBAs often confers sensitivity to one or more of the others. This seems to be more frequent with aminosteroid- rather than with benzyloquinoline-derived NMBAs (3, 17). Therefore, cross-sensitization among different agents has been reported to be frequent, varying between 60 and 70% of patients allergic to NMBAs. While some pairings are common, the patterns of cross-reactivity vary considerably between patients. In fact, it is unreal that an individual may be allergic to all NMBAs (3).

In some cases, the antigenic determinant may either correspond to the tertiary or quaternary ammonium epitope or extend to the adjacent part of the molecule. Cross reactivity can also depend on the structure of NMBAs (flexibility, interammonium distance). Another possibility might be that IgE antibodies could be complementary to structures other than the ammonium group for the relative affinities of the different NMBAs for their IgE antibodies (1).

Since it has been reported that in 15 to 50% of cases, IgE mediated anaphylaxis has been reported at the first known contact with a neuromuscular blocking agent (18). Moneret and coworkers suggested a possible cross-reaction with IgE antibodies generated by previous contact with apparently unrelated chemicals and hypothesized that quaternary and tertiary ammonium ions were the complementary allergenic sites on the reactive drugs. This is a particularly attractive hypothesis in cases where patients react to relatively small and ubiquitous epitopes such as substituted ammonium groups. Indeed, these structures occur widely in many drugs but also in

foods, cosmetics, disinfectants and industrial materials. However, there is no evidence to support the suggestion that allergy to cosmetics sharing structural similarities predisposes to reactions to NMBAs (1).

Delayed immune mediated hypersensitivity reactions

Only few reports concern delayed allergic reactions to NMBAs. T cell mediated immune responses play an important role in both immediate and delayed types of adverse drug reactions. Recently, a delayed hypersensitivity to suxamethonium, driven by an oligoclonal T helper cell 1- skewed CD4+ memory T cell population, expressing the skin homing receptors CLA (cutaneous lymphocyte antigen) and CCR4 has been described (19).

Non immune mediated hypersensitivity reactions

The rate of non IgE mediated immediate hypersensitivity reactions usually varies between 20 % and 35% of the reported cases in most large series (3). In a recent report, non allergic suspected reactions to NMBAs occurred with almost the same frequency as did those with an allergic component (6). Although the precise mechanisms of these reactions remain difficult to ascertain, they usually result from direct non specific mast cell and basophil activation. Direct histamine release is usually regarded as responsible for the non-IgE mediated reactions.

There is not always a clear correlation between circulating histamine levels and the resulting symptoms, particularly with bronchospasm. Histamine may produce adverse effects during anaesthesia, which can be prevented by the use of H1 and H2 blockers (1). Reactions resulting from direct histamine release are usually less severe than the true IgE-mediated reactions (3), with the exception of a subset of patients who have been considered as "super-responders" to the histamine releasing effect of NMBAs (20). Histamine release per se is predominantly found with the use of the benzyloquinolines, d-tubocurarine, atracurium and mivacurium and the aminosteroid rapacuronium (1).

NMBAs may also cross-react with nicotinic and muscarinic receptors causing peripheral autonomic and ganglionic effects (21). Recently, severe bronchospasm resulting from the administration of rapacuronium was reported in children. There were no significant differences between patients with and patients without bronchospasm with respect to age, gender, physical status, history of drug allergy, rapacuronium dose, use of morphine (22). It has been suggested that the higher affinity of rapacuronium for M2 versus M3 muscarinic receptors could account for the high incidence of bronchospasm observed in clinical practice. As a result of these adverse reactions,

rapacuronium has been withdrawn from the market in the USA (23).

Bronchospasm has also been reported with the use of other NMBAs including succinylcholine, d-tubocurarine, atracurium, rocuronium, mivacurium (24). Paediatric patients with bronchospasm during induction anaesthesia were 10 times more likely to have received rapacuronium than another neuromuscular blocking drug (25).

CLINICAL FEATURES

Immune and non-immune mediated reactions to NMBAs cannot be distinguished on the basis of clinical symptoms alone, because clinical symptoms can be very similar and the intensity of reactions shows striking variations from one patient to another (2).

Anaphylactic reactions may occur at any time during the perioperative period, but, in the case of hypersensitivity to NMBAs, they are observed early after induction of anaesthesia, during the induction and maintenance phases, within minutes or seconds after injection of the responsible drug (1, 8). Generally, all early symptoms usually observed in the awake patient such as malaise, pruritus, dizziness and dyspnoea are absent in the anesthetized patient.

Manifestations can range from mild non-life threatening reactions to severe shock and death. The severity of the anaesthetic reactions was based on the grading system for generalized hypersensitivity reactions by Brown (26). This classification is used not only for NMBAs anaphylaxis but for all anaphylactic reactions during anaesthesia, ranging from grade 1 that include skin/subcutaneous involvement to grade 4 that include cardiac and/or respiratory arrest (26).

Mertes and coworkers observed that cutaneous symptoms (e.g. erythema, urticaria and oedema) occur in 65.4% of IgE mediated reactions, whereas these symptoms were present in 94.8% of non-IgE mediated reactions (1). On the contrary, NMBA-induced cardiovascular symptoms, i.e. hypotension, cardiovascular collapse, bradycardia and cardiac arrest, were present in 90.7% of IgE mediated reactions, while only in 36.4% of non-IgE mediated. Bronchospasm occurred more frequently in IgE mediated reactions than in non-IgE mediated reactions (43.4% versus 19.3%) (1).

Suxamethonium, vecuronium, pancuronium and atracurium were reported as linked to adverse drug reactions in children less than 1 year of age. Unfortunately, the Yellow Card Scheme, the main reporting system for adverse drug reactions in the U.K, does not record ages in detail to provide, for example, neonatal adverse responses (6).

The differential diagnosis of any adverse reaction

during or following anaesthesia should exclude the possibility of other conditions that can simulate anaphylactic reaction, e.g. malignant hyperthermia, pulmonary oedema (1).

INVESTIGATIONS

Every patient who experienced a hypersensitivity reaction during anaesthesia should benefit from immediate and/or delayed investigations to confirm an eventual IgE mediated allergic reaction, to identify the responsible drug and to provide recommendations for future aesthetic procedures (27).

In the absence of any gold standard, such as a challenge test, the performance of the various diagnostic methods used to investigate sensitization against NMBAs is difficult to ascertain. The diagnostic strategy is based on a detailed history and on a combination of investigations performed both immediately and days to weeks after the adverse event (see fig. 1) (1).

The starting point of investigation is the history of the reaction. This is provided in the anaesthetic record, drug charts and any additional description or note from the anaesthetist. It is essential to identify for a likely cause the timing of the reaction in relation to events, i.e. induction, start of surgery, administration of other drugs, i.v. fluids, etc. A detailed medical history is necessary because pre-existing asthma may be the only cause of a bronchospasm event during general anaesthesia. Time of onset and clinical features indicate likely causes (anaphylaxis to NMBAs occurs within minutes of administration).

The only real documented risk factor for allergic reaction to NMBAs is a history of a severe undiagnosed adverse event during previous anaesthesia, which should be investigated with preoperative skin testing before undergoing elective surgery (27). Therefore, if the appropriate documentation is available, it should be possible from the history to identify a short list of likely causes (28).

Various tests, including skin testing (prick and intradermal testing) and biological investigations, can be used to confirm the diagnosis of anaphylaxis. In most reports, skin tests (immediately or better 6 weeks later) in association with history remain the mainstay of the diagnosis of an IgE-mediated reaction (1).

In vitro analyses include mediator release assays at the time of the reaction such as basophil activation assays (histamine and tryptase release, anti-CD203c and CD63) and quantification of specific IgE (29).

Early tests are essentially designed to determine whether or not an immunological mechanism is involved. Delayed testing attempts to identify the responsible drug. Whenever possible, confirmation of the incriminated

allergen should be based on immunological assessments using more than one test.

Skin prick and intradermal testing

The use of skin prick tests (SPTs) to verify the anaphylactic aetiology of an adverse event during anaesthesia, establishes the drug responsible and predicts the safety of alternative drugs (1).

The aim of skin testing is to demonstrate specific IgE antibodies. Drugs used for induction of anaesthesia cannot be re-administered; hence, positive skin tests can never be fully validated. SPTs are usually carried out 4 to 6 weeks after a reaction, because prior to 4 weeks the intracellular stocks of histamine and other mediators are still lower than normal. Skin tests to NMBAs may remain positive for years later (30). Although highly reliable, skin tests are not infallible (1). Standardized procedures and dilutions must be precisely defined for each tested agent in order to avoid false positive results due to any direct histamine releasing properties as it is the case for known histamine-releasing compounds such as mivacurium, atracurium and tubocurarine (2, 27).

Administration of mivacurium, atracurium and tubocurarine in fit, non allergic patients was associated with a 370%, 234% and 252% increase in plasma histamine concentrations at 1 min, respectively. Corresponding increase at 3 min were 223%, 148% and 157%, respectively. The changes in plasma histamine concentrations after rocuronium or vecuronium were not significant.

There was no relationship between plasma histamine concentration after mivacurium, atracurium and tubocurarine and cutaneous manifestations (31).

A certain degree of controversy remains as to the maximal concentrations to be used both for SPTs and for Intradermal Tests (IDTs) (32). The British recommendations are that SPT to anaesthetic agents should be at two concentrations: "neat" (i.e. stock solution as used clinically) and at 1/10 dilution, simultaneously. The 1/10 dilution is used to reduce false-positives from drugs with intrinsic histamine-releasing activity (28). A positive weal to neat, but negative to 1/10 dilution, may be considered diagnostic in some circumstances if the drug fits the clinical picture and other possible drugs are ruled out.

Detailed recommendations for SPTs dilutions of anaesthetic drugs including NMBAs have recently also been proposed by the SFAR (Société Française d'Anesthésie et de Réanimation) and SFAIC (Société Française d'Allergologie et d'Immunologie Clinique) (27) (Table 1). The French Society has recommended that drug dilution can be stored for up to 3 months at 4°C, except for atracurium, rocuronium, mivacurium and cisatracurium,

which should be freshly diluted (1/10 dilution) (1, 27).

It is not possible to validate positive SPTs to NMBAs or i.v. anaesthetics using the gold standard of incremental challenge. Therefore, the sensitivity of a positive skin test to i.v. anaesthetics and NMBAs remains unknown and validation is based on correlation of a positive SPT with the clinical picture and restricted to drugs for which there are extensive data.

However, according to some authors, the estimated sensitivity of skin tests for muscle relaxants is approximately from the 94 to the 97 % (1). In a study considering several drugs, NMBAs showed good positive and negative predictive values for SPTs for suspected drug allergy (33). Positive SPTs to NMBAs not administered at the time of the reaction are identified when skin testing is carried out to a wide range of drugs (34).

If no cause can be identified, one cannot be certain if this was due to a false-negative skin testing, which may re-expose the patient to the same or a related NMBAs (1).

IDTs should be undertaken if SPTs are negative for a drug suspected to be the cause and the mechanism of reaction to that drug is such that intradermal testing is appropriate. Like for SPTs, there are also difficulties in interpretation of IDTs, since intrinsic histamine releasing activity is even more marked than in SPTs. However, some studies have reported similar diagnostic values and up to 97% concordance between IDTs vs SPT for NMBAs (35).

Atracurium, mivacurium and rocuronium may result in false positive intradermal reactions in normal subjects at 1/100 of therapeutic concentrations; therefore, lower initial concentrations may be necessary (27, 32). In fact, according to Berg and coworkers (32), more than 90% of the individuals with low potential for allergic reactions will have a non-mast-cell related IDT elicited by the muscle relaxants rocuronium and cisatracurium.

In the United Kingdom, the practice is to conduct IDTs to NMBAs at the highest concentration that does not cause a reaction in normal subjects. In France, the practice is to start at very low concentrations and then to continue at 10-fold increments. Negative SPTs are often found and therefore IDTs are required at concentrations ranging from 1/100 dilution to the therapeutic concentration (Table 1).

IDTs to NMBAs are associated with a higher risk of systemic reactions, while SPTs even in patient with anaphylaxis appear to be safer. The prevalence of SPTs induced anaphylaxis has been estimated to be less than 0.3% (31). Therefore, when administering a NMBA to a sensitized patient with negative SPTs one should bear in mind the risk-benefit ratio. In addition any new muscle relaxant should be routinely tested in patients known to be allergic to these agents in order to detect possible cross-

reactions (27).

Cross reactivity, as written before, occurs commonly among NMBAs and can be detected by SPTs, but much higher rates (60-84%) are found by intradermal testing, although only a minority react to all tested drugs (27, 30).

In a patient who presented an allergic reaction to NMBAs, all available neuromuscular blocking agents should be tested (2, 18, 27, 30). In patients with a suspected allergy to NMBAs, IDTs are only required if SPTs are negative or to distinguish cross reacting drugs if an IDT was necessary to identify the causative agent (Fig. 1). If the cause has not been identified on the initial visit, SPTs should be repeated at a later date (28).

Basophil activation assay

During an IgE-mediated reaction, basophils and mast cells are activated and then degranulate and release mediators in intracellular fluids. These mediators can be measured in the patient's serum and have proved to be useful for the diagnosis of anaphylaxis during anaesthesia (3).

There are different categories of mediator release tests: histamine release and tryptase tests.

Histamine concentrations are maximal almost immediately and decrease thereafter with a half-life of about 20 min (1). Therefore, circulating levels should be assayed within the first hour of a reaction, and in mild cases, only the early measurements may be increased. When increased, histamine circulating levels confirm basophil cell activation, which can result from direct or IgE-mediated activation. In a recent study, the sensitivity of this test for the diagnosis of anaphylaxis was estimated at 75 %, the specificity at 51%, the positive predictive value at 75% and the negative predictive value at 51% (1). Mata et al. have evaluated the in vitro leukocyte histamine release (LHR) tests for the diagnosis of allergy to muscle relaxant drugs. These tests were positive in 65% of the allergic patients, for a threshold corresponding to a specificity at 100%. Despite a very good specificity, their diagnostic application remains limited because of the heavy experimental conditions and nonsufficient sensitivity (36).

Tryptase is a mast cell tetrameric neutral serine trypsin, like protease co-released with histamine when mast cells are activated during anaphylaxis. There are two structural forms of tryptase identified by specific antibodies, alpha and beta tryptase that share approximately 90% of sequence homology. Alpha tryptase is a useful marker of a pathologically increased number of mast cells, as observed in mastocytosis. Pro-beta tryptase is secreted constitutively and serves as a measure for mast cell number, whereas mature beta-tryptase reflects mast cell activation, particularly during anaphylaxis. Human

basophils also contain tryptase, but their levels are 300 to 700-fold lower than in skin or lung mast cells.

Unlike histamine, which has a half-life of very few minutes, the beta tryptase has a half-life of 120 minutes. Tryptase reaches a peak in the patient's serum 30 minutes after the first clinical manifestations, but sampling is recommended 60-120 min after onset of symptoms. Its levels usually decrease over time, but in some cases elevated levels can still be detected for up to 2 days or more after the onset of anaphylaxis (1). Thus, although elevated tryptase levels can be observed in different situations, an elevated tryptase concentration ($> 25 \mu\text{g/L}^{-1}$) is usually regarded as specific for mast cell activation and differentiates between an IgE-mediated and alternative effector cell activation (37). However, the absence of increased serum tryptase does not rule out an allergic reaction (38). False negative results have been attributed to mechanisms where the reaction involves basophils rather than mast cells (1), whereas false positive results have been reported in cases of extreme stress such as hypoxemia and major trauma. In absence of anaphylaxis, tryptase remain stable and does not vary by more than 2.0 microgram/L in any given individual over a short timeframe. It could be useful to measure tryptase levels in baseline, 15' and 60' minutes serum samples. Measuring changes in tryptase from baseline significantly improved both sensitivity and specificity, as tryptase levels appear to be stable in the absence of any allergic reaction (39).

Indeed, Malinowski and coworkers demonstrated that neither a modification of the actual diagnostic threshold nor the determination of tryptase values the day following an adverse reaction will increase the diagnostic value in a clinically relevant manner (40).

In some reports, using tryptase measurements for the diagnosis of anaphylaxis, its sensitivity was estimated at 64 %, specificity at 89.3 %, positive predictive value at 92.6 % and negative predictive value at 54.3 % (3). As expected, increased tryptase value is a strong argument to support the diagnosis of IgE mediated hypersensitivity reactions. However, despite an excellent positive predictive value, the relatively low sensitivity of tryptase measurement cannot support its use as sole screening tool to determine the need for a specific allergological determination.

Unfortunately, since the only RIA (Radioimmunoassay) commercially available measures both tryptase isoforms, it is often not possible to distinguish the two isoforms.

Flow cytometry can contribute to the diagnosis of hypersensitivity reactions from NMBAs. The basis of flow-assisted allergy diagnosis relies on quantification of shifts in expression of basophilic activation markers after challenge with a specific allergen using specific antibodies conjugated with a fluorochrome or a dye. Basophils from

allergic patients produce different cytokines immediately after the appropriate allergen challenge. Currently, the most commonly used antibodies in allergy diagnosis are anti-CD63 and, to a lesser extent anti-CD203c (41). CD63 is anchored in the basophilic granule membrane (which contains histamine) and its exposure to the outside of the cells reflects cell degranulation, due to fusion between granule and plasma membranes. Thus, CD63 expression has been proposed as a reliable means to monitor basophil activation (42). The expression of CD63 seems to be more closely related to anaphylactic degranulation than CD203c (41). Monneret and coworkers concluded that their flow cytometry protocol using CCR3 and/or CD203c was a promising tool in allergy diagnosis, even if its sensitivity needs to be improved. They found sensitivity for CD63 test similar to that of specific IgE detection and higher than the one of histamine release test (43).

Abuaf and coworkers showed that the sensitivity and specificity of a CD63-based basophil activation assay were 64 % and 93 %, respectively.

Serum -specific IgE Assay

In vitro tests are available to detect the presence of serum specific IgE antibodies.

The detection of anti-drug specific IgE assays in serum is performed by a sandwich-type immunoassay in which the serum IgE is first adsorbed to a reactive phase and subsequently quantified via the binding to an anti IgE tracer. The reactive phase is prepared by covalently coupling a drug derivative to a solid phase such as nitrocellulose membrane or a polymer. Baldo and Fisher were the first to demonstrate that drug reactive IgE were involved in anaphylactic reactions, using NMBA's coupled to epoxy Sepharose in a radioimmunoassay (44).

PAPPC RIA test (P-aminophenyl phosphoryl choline radioimmunoassay) was developed by Guilloux and coworkers for the detection of specific IgE directed against tertiary and/or quaternary ammonium groups of NMBA's in sera of subjects sensitized to these drugs (40).

P-aminophenyl phosphoryl-choline contains a larger choline derivative (quaternary ammonium ion) including a secondary ammonium group, an aromatic ring and a phosphate group. P-aminophenyl phosphoryl-choline immobilized on cross-linked 6% beaded agarose was used in different studies. The test appears to be the most efficient test to screen sera for the presence of IgE antibodies to ammonium groups of NMBA's. Guilloux and coworkers have reported 97% specificity and 94% positive predictive value of the PAPPC RIA (40). No inhibition test has been done to detect the specificity of the antibodies detected by the PAPPC-RIA.

Morphine, which has a single substituted ammonium group, avidly binds in vitro to antibodies that react to

NMBA's. Morphine chloride was coupled to Epoxy-activated Sepharose according to procedures described by Harle and coworkers (44).

The mechanism of this reaction has been shown to be a cross-reactive recognition of substituted ammonium groups which are shared by NMBA's, morphine and a number of other drugs. The morphine RIA has proved to be a sensitive and efficient test for the detection of IgE to NMBA's and Fisher and Baldo have reported a specificity of 98% and a sensitivity of 85% for the test. The test offers an improvement of the test battery of RIAs for individual NMBA's, where the sensitivity is reported to be as low as 52% (45).

Choline chloride was coupled to Epoxy-activated Sepharose and used to detect IgE antibodies that react with suxamethonium, but this test showed a low sensitivity. However, some patients do not react with all NMBA's, showing that the substituted ammonium ion is at least not always the only part of epitope.

IgE binding on different NMBA solid phases and competitive inhibition assays with several muscle relaxants and other drugs demonstrated a cross-reactivity of specific IgE (1).

More recently, Ebo and coworkers investigated the diagnostic value of quantification of IgE by ImmunoCAP in the diagnosis of rocuronium allergy (46). They concluded that the rocuronium ImmunoCAP constitutes a reliable technique to diagnose rocuronium allergy, provided an assay-specific decision threshold is applied, because these assays reach a sensitivity of more than 85% and an absolute specificity.

In clinical practice, immunoassays for the detection of drug reactive IgE may provide important information to confirm the responsibility of suspected drug (3, 36). This test is usually performed several weeks after the reaction but can be carried out at the time of the reaction (1).

MANAGEMENT

The potential severity of anaphylaxis to NMBA's underscores the interest of developing a rational approach to reduce its incidence by identifying potential risk factors before surgery. However, in the absence of an established predictive value of tests for the occurrence of anaphylactic reactions, there is no demonstrated evidence for systematic preoperative screening in the general population at this time (1, 8). The aim of investigation is to identify cause of anaphylaxis, to identify and avoid cross-reacting NMBA's and to clarify the list of drugs likely to be safe for future use, but to get it, there isn't a gold standard. There are two types of analysis useful in the investigation (Fig. 1): biological and "in vivo" tests, but both tests must be put together with history that include

clinical notes (risk factors), anaesthetic record (type, time and features of reaction).

Although several risk factors occur more frequently in patients with anaphylaxis during anaesthesia, their prevalence in the general population is such that few would benefit in terms of preoperative screening, especially when anaphylactic reactions to NMBAs are concerned.

Sex and age cannot be considered as true risk factors since reactions to NMBAs have been reported at all ages, including children (3).

A family history of anaphylaxis has been suggested as important in one report of cousins who reacted to neuromuscular blocking agents but no similar cases have been reported in the literature. In many large series of patients sensitized to a NMBA a disproportionate number of patients have histories of atopy, allergy or asthma (1). However, other studies have failed to demonstrate an increased risk of allergy to these drugs in atopic patients (3). Similarly, neither a history of allergy to other drugs, chronic fatigue syndrome nor multiple chemical sensitivity predispose to anaphylaxis during anaesthesia (8). There is no evidence of increased risk of anaphylaxis to NMBAs in patients who have had anaphylaxis to drugs not used in the operating theatre (1).

Since a first exposure to an antigen is considered as a prerequisite for the development of a sensitization, it has been suggested that patients who have previously been exposed to a NMBAs may constitute a high-risk group. However, the highest incidence of prior exposure to a muscle relaxant in patients allergic to these is around 50%. It is apparent that under some circumstances IgE antibodies against tertiary or quaternary ammonium ions are not formed in response to exposure to a muscle relaxant but to an as yet unidentified antigen. Thus, in clinical practice, the use of a history of previous exposure to NMBA as a potential risk factor may preclude the identification of at least half of potential reactors (18). Consequently, pre-anaesthetic assessment of sensitization to NMBAs should be limited to the following high risk patients:

- Patients presenting a documented allergy to muscle relaxants.
- Patients having experienced an unexplained reaction during a previous general anaesthesia, including severe hypotension, bronchospasm, cardiac arrest, wide spread rash, flushing, urticaria or oedema during recovery. If the anaesthetic protocol is unavailable, the substances (i.e., muscle relaxants and latex) that are most often incriminated in epidemiologic studies should be tested (skin tests, eventually specific IgE assays). One should keep in mind, however, that skin tests performed belatedly after an adverse reaction during anaesthesia could be falsely negative. This is due to a possible spontaneous

decrease of specific IgE concentration with time. It is always preferable that any investigation be carried out at least 6 weeks after the adverse reaction.

It is highly recommendable to perform SPTs with all the commercially available NMBAs. On the one hand, since skin tests with muscle relaxants remain positive for several years, sensitization may be identified as a result of previous surgery. On the other hand, because of the high frequency of cross reactivity between NMBAs, testing all the drugs available increases the chances of finding a drug with a negative skin test that could be used as alternative. Cross-reactivity to NMBAs was found in most of sensitized patients. The use of a drug with a negative SPT was recommended as an alternative, according to the results of previous publications (35).

If tests are negative, equivocal or unexpected, it is necessary to repeat tests and consider higher concentration. With both normal tryptase level and negative skin tests, one can be fairly certain to reject the diagnosis of IgE mediated hypersensitivity reactions, but not on a normal tryptase value alone. In addition, an increased tryptase concentration remains a strong argument for the diagnosis of IgE mediated hypersensitivity reactions when skin tests could not be performed (1).

PREVENTION

Prevention of anaphylaxis has two major objectives: 1) preventing sensitization of a patient to a particular allergen, or 2) preventing the occurrence of an anaphylactic reaction to a re-introduced allergen in a pre-sensitized patient. In this regard, in the wake of the relatively high rate of sensitization in the absence of any prior exposure, optimal prevention of sensitization to NMBAs, even if their administration were to be radically curtailed, is probably unattainable.

Prevention of anaphylactic reactions relies mainly on accurate documentation of previous reactions and avoidance of the incriminated drug.

Pre-treatment with corticosteroids or histamine-receptor antagonists, by either H1- or H1- and H2-receptor antagonists remains controversial. No evidence of beneficial effects of prophylactic administration of corticosteroids in anaesthesia has been shown at this time. Pre-treatment with H1- and H2-receptor antagonists has been found to reduce histamine mediated adverse effects in various studies. Antihistamine administration was effective in reducing the adverse effects of non-immune histamine release following muscle relaxant (1). However, histamine detected during alarming immune-mediated reactions is merely a marker of co-release of more dangerous mediators. In addition, such a prophylactic approach has been found to be ineffective,

or, in some cases, deleterious (8). Many authors consider that pre-treatment with corticosteroids, or antihistamines, or both, do not provide reliable prevention of immune-mediated reaction. Nevertheless, even in the absence of any well-documented studies concerning anaphylaxis, some authors propose pre-treatment with H1 or H1 and H2 antagonists as useful in the management of the patient with a history of anaphylaxis or at risk of non-immune histamine release (1).

In France, the use of these associations remains a matter of controversy. When prescribed, preventive treatment is usually limited to H1-receptor antagonists. However, proven anaphylactic reactions to NMBAs, even in the wake of preoperative H1- and H2-receptor antagonists and steroids, have been documented in epidemiologic surveys (8, 17).

The use of monovalent haptens, which can occupy antibody sites without bridging specific IgE fixed on sensitized cells, has also been proposed for muscle relaxants. In this respect, any molecule presenting a quaternary ammonium ion could be considered as a potential monovalent hapten.

Choline and tiemonium were initially used; unfortunately, clinical tolerance of the highest doses was poor. As a result, the concentrations obtained were too low to be effective (1). Recently, Moneret-Vautrin and coworkers demonstrated inhibition of skin mast-cell reactivity to muscle relaxants by mixing them with the monovalent haptens cytidylcholine and ethamsylate.

Furthermore, they obtained an inhibition of leukocyte histamine release for up to 3 hours following the infusion of these monovalent haptens in patients allergic to muscle relaxants. Morphine, with its high affinity to reactive muscle relaxant antibodies, has also been proposed as a possible preventive hapten. However, prevention of muscle-relaxant anaphylaxis by monovalent haptens cannot be recommended in standard clinical practice (1).

CONCLUSIONS

Among all drugs used for general anaesthesia, NMBAs seem to play a predominant role in the incidence of severe adverse reactions. Since their introduction in modern anaesthesia practice, much progress has been made in the identification of adverse reactions and their potential mechanisms. However, despite the introduction of newer drugs in clinical practice, the incidence of serious, immediate hypersensitivity-type reactions has remained virtually unchanged.

A gold standard for the diagnosis of anaphylaxis has not been defined. Because no premedication can effectively prevent an allergic reaction, any suspected anaphylactic reaction must be extensively investigated

using combined perioperative and postoperative testing. Patients must be fully informed of the results of the investigations, and advised to provide a detailed report prior to any future anaesthesia. A medical alert bracelet or a warning card is strongly indicated for these patients.

Currently, with the exception of the high-risk patient, a systematic preoperative screening for sensitization against NMBA is not justified. A pre-anaesthetic history is the most important tool for screening subjects who are at risk of having an abnormal reaction during anaesthesia. Particular attention must be paid to patients who have already experienced such a reaction during anaesthesia, or those alleging an allergy to muscle relaxants. The decision to carry out allergy tests is justified whenever a hypersensitivity reaction occurs during general anaesthesia, to avoid similar problems during subsequent operations. In these cases, the choice of the safest possible anaesthetic agents should be based on the result of a rigorously performed allergologic assessment.

The diagnosis of allergy is mainly based upon an evocative clinical history, positive skin tests which remain the gold standard in this context, and if available, detection of specific IgE (see Fig.1). In the most cases, these features allow both diagnosis and identification of the allergen responsible for anaphylactic reaction. In the remaining cases, especially for drug allergy characterized by many discrepancies between clinical assessment of the disease and biological results, functional *in vitro* studies are needed, such as histamine release (43).

In practice, even if NMBA specific IgE are positive and skin tests negative, some authors recommend that NMBAs are not used again. Regardless of the specific IgE results, NMBAs are contraindicated if the skin tests were positive. In view of the constantly evolving anesthesiologic practices, and of the relative complexity of allergy investigation, an active policy to identify patients at risk and to provide any necessary support from expert advice to anaesthetists and allergologists through the constitution of allergeo-anaesthesia centers should be promoted.

The high frequency of IgE anaphylactic reactions and the feasibility of skin tests in children justify systematic allergy testing whenever a hypersensitivity reaction occurs during general anaesthesia (16).

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