

The role of tryptase assay in recognition of anaphylactic reactions to muscle relaxants

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The incidence of allergic reactions during anaesthesia has been estimated to be from 1:3500 to 1:20000, with muscle relaxants being the most common allergens. Symptoms may involve all organs and systems with various intensities - from mild skin reactions to bronchospasm and cardiovascular collapse in the most severe cases. In a case of suspected perioperative allergic reaction, the management should not be limited to resuscitation and restoration of basic life functions, but must include careful investigation of potential allergens. An appropriate diagnostic strategy is essential for confirmation of an allergic reaction, identification of the allergen, and recommendations for future prevention. In this review, the various aspects of anaphylaxis and anaphylactoid reactions to muscle relaxants are discussed, including the role of tryptase and its assay in the diagnostic regimen.

The incidence of anaesthesia-related allergic reactions varies between 1:3500 and 1:20000 anaesthetic procedures [1, 2]. The relevance of perioperative allergic reactions was particularly realized in Scandinavia, Western Europe, Australia and New Zealand [3, 4]. The anaphylactic symptoms are life-threatening with the mortality rates ranging from 3% to 6%; some of such cases are associated with permanent brain damage [2, 3, 4, 5]. The prevailing allergens in the perioperative period are skeletal muscle relaxants, mostly suxamethonium, atracurium, vecuronium, rocuronium and less commonly - pancuronium, mivacurium and cis-atracurium [6, 7, 8, 9, 10, 11, 12]. Laroche and colleagues [13] observed that the use of muscle relaxants was associated with increased risk of allergic reactions.

Allergic reactions during anaesthesia were described in general, gynaecological, obstetric, maxillofacial and laryngological surgeries [14]. The risk factors included female gender and earlier post-drug reactions [2, 5, 8, 14, 15]. In the adult population, allergic reactions were more common amongst females aged about 40 years and male patients about 10 years older. Karila and co-workers [10] demonstrated that such visible female gender predominance was not observed among children and teenagers in whom allergic reactions were equally common occurring in 1:2100 cases of anaesthesia. Coexistence of atopy and immunological diseases was not associated with increased risk of such reactions. It was stressed, however, that basophiles and mast cells in individuals with coexisting immunological diseases were more sensitive to chemical agents and therefore the drugs directly stimulating inflammatory cells, including muscle relaxants, atracurium and mivacurium, in particular, should be used with caution [5]. Moreover, the cases of familiar anaphylactic reactions to muscle relaxants were reported [16].

PATHOGENESIS OF ALLERGIC REACTIONS TO MUSCLE RELAXANTS

Pathogenetically, allergic reactions to muscle relaxants occur in the form of anaphylactic reactions triggered by indirect, immunological IgE-mediated activation of mast cells or anaphylactoid reactions triggered by direct activation of mast cells by allergens [1]. The pathogenetic mechanisms and course of anaphylactic as well as

anaphylactoid reactions are illustrated in Fig.1.

The effector cells of allergic reactions are basophiles and mast cells. The pathogenesis of their activation is multifactorial. The specific receptor interactions between an allergen and IgE-mast cell complexes lead to changes in spatial conformation of proteins – membrane enzymes (tyrosine kinase), which initiates the cascade of biochemical changes. Increased levels of intracellular calcium induce the release of mediators from granules: histamine, neutral proteases – tryptase, proteoglycans – heparin, chondroitin sulphate. The simultaneous activation of enzymatic intracellular proteins (phospholipase A2 – PLA2, phospholipase C – PLC) induces the metabolism of membrane phospholipids leading to formation of arachidonic acid. The further changes of arachidonic acid mediated by lipoxygenase and cyclooxygenase result in accumulation of mediators synthesized on demand: leucotrienes, prostaglandins and thromboxanes.

Non-immunological activation of mast cells results from direct effects of an antigen or is mediated by complementary binding of complement elements C5a, C3a and C4a with specific membrane receptors. Irrespectively of the mechanism triggering the allergic reaction, activation of mast cells leads to synthesis of the platelet activating factor (PAF), interleukins (IL-4, IL-5, IL-6, IL-8, IL-16), TNF α , interferon γ (IFN γ), which through their interactions enhance the allergic reaction involving other cells, e.g. eosinophils, neutrophils, lymphocytes T, B, dendritic cells, vascular endothelium, blood platelets and myocytes [1].

The pathogenesis of anaphylactic reactions to muscle relaxants is markedly affected by the chemical structure of allergens and sensitization. It was demonstrated that commonly occurring food and chemical allergens, e.g. detergents, some cosmetics, disinfectants, industrial agents containing ammonium groups, induce sensitization with generation of IgE antibodies. Due to their similar chemical structure, resulting from the tertiary and quaternary ammonium groups, skeletal muscle relaxants and allergens interact specifically with the active locus of immunoglobulin E and activate the mast cells [17]. Veien and colleagues [18], who studied determinants of mechanisms of non-immunological activation of mast cells by muscle relaxants, emphasized the pathogenetic importance of PLA2 and PLC.

The use of inhibitors of these enzymes limited the processes of degranulation of mast cells and release of mediators. Moreover, the authors demonstrated that histamine and tryptase were biochemical markers of anaphylactoid reactions. In the general population, anaphylactic reactions were more common than the anaphylactoid ones; amongst children and young individuals, immunological reactions predominated accounting for about 90% of allergic reactions in the perioperative period [8, 10].

CLINICAL SYMPTOMS

Clinical symptoms of anaphylactic and anaphylactoid reactions are similar. They may be local or systemic and develop very rapidly – in 86% of cases they occurred 5 min after the exposure to an allergen. Circulatory and respiratory manifestations predominate in anaphylactic reactions whereas skin symptoms in anaphylactoid reactions. Ring and Messmer [19] introduced the 5-grade scale of severity of allergic reactions (Table 1). Anaphylactoid reactions mostly induce the grade 1 symptoms while in anaphylactic ones the grade 2 and 3 symptoms are most commonly observed [4, 8, 9, 10]. The presence of risk factors was not found to affect the severity of allergic reactions [5].

The critical stage of general anaesthesia is its induction with muscle relaxants. In order to detect the allergic reaction during anaesthesia, it is essential to observe the clinical symptoms and assess the stability of vital parameters. The basic markers of dysfunctions of individual organs and systems during allergic reactions are presented in Table 2 [13].

Muscle relaxants trigger allergic reactions immunologically and non-immunologically; most commonly they cause the symptoms classified as grade 3 of severity [6]. The cross reactivity of muscle relaxants is common

[5, 6, 20]. It was demonstrated that in 60% of patients with anaphylactic reactions related to a particular muscle relaxant, skin tests were also positive to other preparations of the same chemical group. The occurrence of cross reactivity was associated with the similarities in chemical structure [2, 17, 21].

DIAGNOSTIC METHODS

The main diagnostic methods include: biochemical (determination of concentrations of allergic mediators), serological (detection of specific IgE antibodies) and skin tests. Moreover, detection of activated mast cells (based on membrane particles CD63, CD203a) using flow cytometry or the test for release of histamine from leucocytes are applied [22, 23, 24, 25, 26].

In biochemical diagnostics of allergic reactions, tryptase and histamine are most relevant mediators. Moreover, monitoring of concentrations of serotonin, eosinophil cation protein, leucotriens and prostaglandins was also described yet they are not specific for allergic reactions and their determinations are not routinely performed [5, 13].

Serological examinations and skin tests with the supposed allergens are performed later (skin tests 4-8 weeks after the occurrence of allergic reactions) and hence their diagnostic usefulness in the immediate perioperative period is limited. Biochemical diagnostics can be carried out in the biological material (plasma, serum) collected during anaesthesia and is the only method for early confirmation of the diagnosis. Amongst numerous chemical substances involved in allergic processes related to the use of skeletal muscle relaxants, tryptase is considered essential; according to the Scandinavian Clinical Practice Guidelines, determinations of this enzyme are the basic element of diagnostics of anaphylaxis [4]. The confirmation of anaphylactoid/anaphylactic reactions associated with muscle relaxants based on the presence of plasma tryptase shows accordance with the diagnosis resulting from skin and serological tests. The diagnostic procedures involving serological examinations, skin tests and biochemical determinations of tryptase enable to confirm the allergic reaction, to identify the allergen and to institute secondary prevention.

TRYPTASE

Tryptase is the major mediator of immunologically and non-immunologically-triggered allergic reactions. Genes responsible for the structure and synthesis of tryptase are located on the short arm of chromosome 16. Several varieties of this enzyme were described based on the differences in amino acid composition. In human mast cell granules, β II-tryptase predominates, which occurs as β -preprotryptase – an inactive tetramer proenzyme. The precise mechanisms regulating the tryptase activity have not been elucidated. The effects of action result from the reactions between tryptase and protease activated receptors (PAR-2). PAR-2 are present in the endothelium and smooth muscle layer of the upper and lower airways, pneumocytes, endothelium and muscle layer of blood vessels, as well as in erythrocytes. The activation of PAR-2 is mediated by PLA2 and PLC, which leads to initiation of arachidonic acid metabolism.

The multidirectional effects of biological action of β -II tryptase were described. It causes the breakdown of mediators dilating the bronchi, which may cause the constriction of the smooth muscle layer of airways. In the blood vessel endothelium, nitric oxide-dependent dilation of vessels and their increased permeability with the features of hypoxia were observed. Tryptase was active towards plasma clotting factors (breakdown of fibrinogen, activation of pre-calicrein, breakdown of kininogens, activation of pro-uPA) and finally showed anticoagulative effects [20, 27]. The release of tryptase from mast cells depended on the availability of intra- and extracellular stores of calcium and magnesium as well as the presence of PLA2 and PLC inhibitors [18, 28].

TRYPTASE ASSAYS

Determinations of tryptase in body fluids may be used to assess the allergenic action of muscle relaxants. It was demonstrated that suxamethonium did not induce the release of this substance from both basophiles of peripheral blood and skin or lung mast cells. Otherwise, with atracurium and vecuronium, the effects of tryptase release was directly proportional to the dose used and the dynamics of concentration changes affected by stimulation with aminosteroid and benzyliisoquinolone agents was similar. The effects of atracurium on the release of tryptase from lung mast cells were highly individual. Amongst the agents studied, only atracurium caused the release of histamine from atrial mast cells. Intradermal stimulation of mast cells with both muscle relaxants belonging to the aminosteroid group and benzyliisoquinolones caused a dose-dependent biochemical effect (tryptase release) and increased objective (erythema) and subjective (pain, pruritus) manifestations. With suxamethonium and benzyliisochinolones, subjective symptoms of intradermal stimulation (pruritus, erythema, pain) occurred and intensified with an increase in tryptase concentration, which was not observed with aminosteroids [28, 29].

Furthermore, tryptase assays are used to confirm allergic reactions. Tryptase is essential amongst many allergic markers and assets of its determinations were widely emphasized. Another biochemical marker of allergic reactions to muscle relaxants is histamine yet there are many factors which limit its determinations as a practical marker of allergic reactions. Histamine is present both in basophiles and in mast cells and similar mechanisms induce its release. Thus, it is impossible to determine its source. Due to the dynamics of histamine concentration changes (biological half-life $T_{1/2}$ is about 5-10 min), blood should be sampled directly during allergic symptoms, which is often infeasible. Tryptase is present in mast cell granules and in basophiles, although in trace amounts. This enzyme occurs in complexes with heparin and is characterized by high molecular weight compared to histamine (about 140 kDa) thus, it occurs in body fluids later than histamine. Thanks to dynamics of tryptase changes (half-life - $T_{1/2}$ - 2-3 h), the use of its determinations is time-real and useful [13, 30, 31, 32]. "Postmortalis" determinations of tryptase are also used for the diagnosis of anaphylactic reactions in fatal cases. Some cases were described in which results of tryptase determinations in the biological material collected on day 3 after death revealed anaphylaxis [20].

A variety of laboratory methods for tryptase determinations in body fluids were designed. The radioimmunoenzymatic assay was found most useful. This method enables the detection of β II-tryptase specific for allergic reactions in the concentrations lower than $1 \mu\text{g L}^{-1}$ [20]. It was shown that in the interval up to 6 h after the onset of anaphylactic symptoms there was no dependence between the time of blood sampling and recorded tryptase concentration [20, 33]. Thanks to the kinetic parameters and stability of the enzyme under various environmental conditions, the biological material may be collected and tryptase quantified not only on the occurrence of allergic symptoms. It is recommended to collect blood in the amount of 5-10 mL during 1-4 h following the onset of allergic symptoms [4, 27, 28, 31].

Perioperative allergic reactions are a relevant anaesthesiological problem, as the most common cause of their development is the action of agents used for general anaesthesia. The highest risk of allergic reactions is associated with the induction of anaesthesia and skeletal muscle relaxants are the prevailing allergens. Aminosteroids trigger allergic reactions more frequently than benzyliisoquinolones, accounting for about 50% of cases: rocuronium -30%, vecuronium -17%, pancuronium -6%. In the group of benzyliisoquinolones, allergic reactions were most common after atracurium and mivacurium, 20% and 6%, respectively. The lowest number of allergic reactions was related to cisatracurium - 0.3%. The incidence of allergic reactions to depolarizing muscle relaxants (suxamethonium) is comparable to that related to non-depolarizing aminosteroids [5].

The severity of allergic reactions to muscle relaxants greatly varies: from mild skin symptoms to anaphylaxis, which directly threatens life. Primary prevention is an important aspect of anaesthesiological management - detection of risk factors and institution of actions to reduce the risk of allergic reactions.

It is difficult to determine the risk of allergic reactions associated with anaesthetic agents as such situations most commonly occur in patients who earlier did not undergo anaesthesia. The similarity in chemical structure

of widely present food and industrial allergens leads to sensitization and production of antibodies, which also react specifically with relaxants. Therefore, already the first contact with a relaxant may induce symptoms of the allergic reaction. Moreover, allergic cross reactivity is relevant, which concerns not only skeletal muscle relaxants but also occurs between muscle relaxants and other chemical substances used in the perioperative period, e.g. opioids, antibiotics, colloids, latex. When the allergic reaction during anaesthesia is suspected, three basic diagnostic examinations are essential: biochemical, serological and skin tests, which are necessary to identify the allergen and to institute appropriate anaesthesiological management for secondary prevention. Tryptase determinations are the reliable and the best method to confirm the anaphylactic reaction.

The problem of allergic reactions associated with muscle relaxants is extremely complex due to their multi-directional effects. The issue in question is topical and epidemiological data presented in numerous publications evidence its relevance.

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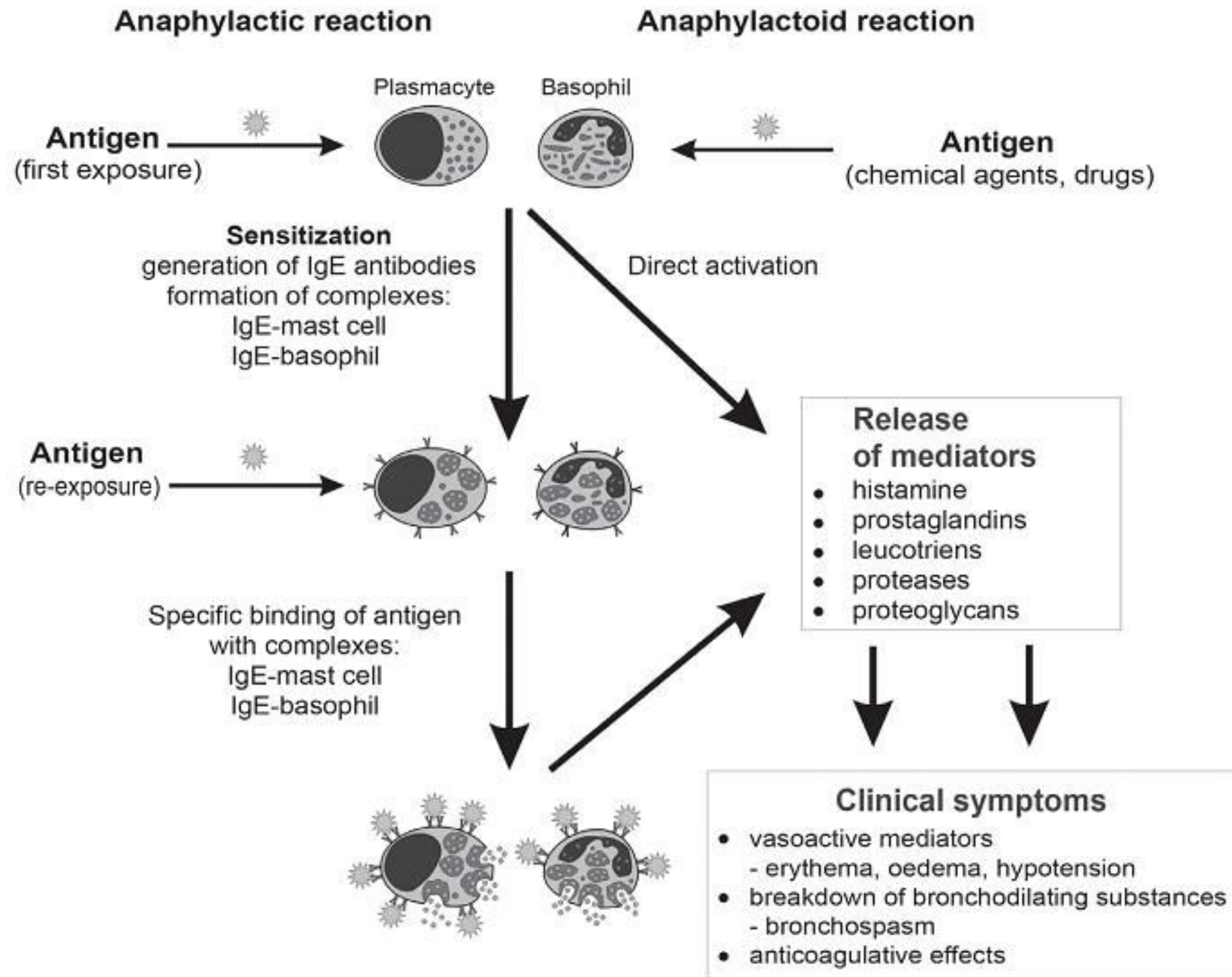


Fig. 1. Pathogenesis of anaphylactic and anaphylactoid reactions

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Grade	Symptoms
1	skin lesions: generalized erythema, urticaria, oedema
2	symptoms not threatening life: skin lesions, decreases in arterial blood pressure, increased heart rate, respiratory abnormalities, cough, inspiratory dyspnoea
3	life-threatening symptoms: shock, tachycardia or bradycardia, arrhythmias, bronchospasm
4	severe circulatory and/or respiratory insufficiency
5	death

Table 1. Severity of allergic reactions according to clinical symptoms

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Organ/system	Symptoms
Circulatory	decreased arterial pressure, increase heart rate, arrhythmias, cardiac arrest
Respiratory	increased peak inspiratory airway pressure, increased end-expiratory CO ₂ , reduced haemoglobin saturation with oxygen, bronchospasm, additional respiratory murmurs above lung fields
Excretory	oliguria
Digestive	lack of or slowed down peristalsis (difficult to observe in a patient undergoing general anaesthesia)
Skin	erythema, local or systemic oedema, urticaria
Haemostasis	vascular clotting syndrome

Table 2. Markers of dysfunction of individual systems and organs in the course of allergic reaction during general anaesthesia