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Tryptase in Diagnosing Adverse Suspected Anaphylactic Reaction*

Tryptaza w diagnostyce reakcji niepożądanych podejrzanych o anafilakcję podczas znieczulenia oraz w okresie okołooperacyjnym

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Abstract

Determination of serum mast cell tryptase (MCT) is becoming more widely used in diagnosing allergic reactions involving mast cells. It can help evaluate the allergenic effects of drugs administered during anesthesia and the perioperative period. Until now, data about the role of tryptase in the body has not been clarified yet. Patients with elevated MCT levels should undergo further testing to find out the causative agent of a potential allergic reaction. Patients with normal tryptase concentration should also undergo further diagnosis if they manifest clinical symptoms of a severe anaphylactic reaction (Adv Clin Exp Med 2012, 21, 3, 403–408).

Key words: tryptase, anaphylaxis, anesthesia, perioperative period.

Streszczenie


Słowa kluczowe: tryptaza, anafilakcia, znieczulenie, okres okołooperacyjny.

A huge increase in allergic diseases observed in the last ten years significantly affects those with allergic hypersensitivity to drugs administered during the perioperative period and anesthesia. According to data presented in the reference literature, the incidence of anesthesia-related anaphylaxis ranges from 1:3500 to 1:20000 anesthesias [1, 2]. Despite the lack of precise epidemiological reports from different regions, the growing risk of anaphylactic reactions in the perioperative period has been thoroughly observed. The importance of the emerging problem of anesthesia-related anaphylaxis was particularly noticed in the Nordic countries, Western Europe, Australia and New Zealand [3, 4].

In 1960, the properties of mast cell granules termed trypsin-like activity were discovered. The isolated enzyme was named mast cell tryptase: MCT [5]. Until now, data on the biological role of tryptase in the body has not been definitively identified and clarified. At the same time, its function in diseases involving mast cells has not yet been fully explained. This results from the fact that the physiological substrate which would react with tryptase has not been discovered yet. Such an ability for some proteins has been demonstrated only

* This article is based on the Medline database from the years 1991–2010. When collecting literature, the author used the following key words: tryptase, mast cell, anaphylaxis, mastocytosis.
under in vitro conditions. In the diagnosis of anaphylactic reactions, tryptase has been used as an enzyme that monitors mast cell activity.

There are many chemicals involved in allergic processes associated with the use of neuromuscular blocking agents (NMBA) and other drugs administered in the perioperative period. Many different drugs are used in rapid succession: not only anesthetics, but also antibiotics, fluids, nonsteroidal anti-inflammatory drugs and other compounds (e.g. disinfectants, latex, chlorhexidine, etc.). Most of these drugs are given intravenously and in bolus, bypassing the body’s primary immune filters and presenting high concentrations of antigen directly to the mast cells and basophils. So it is difficult to say which drug caused the suspected anaphylactic reaction or if the reaction was the result of the additive side effects of several drugs injected simultaneously [1]. Tryptase, however, is perceived to be of the highest importance. According to Scandinavian authors, determining the presence of this enzyme is a key element in diagnosing anaphylactic reactions [4, 5]. The NMBA-related anaphylaxis confirmed by the presence of tryptase in plasma is compatible with a diagnosis resulting from skin and serological tests.

**Mast Cell**

A mast cell (mastocyte, MC) is derived from multipotential bone marrow cells CD34+. It appears in the peripheral blood, and then is deposited in tissues such as skin, lungs, intestinal mucosa and submucosa. There it matures under the influence of general and local growth factors, such as the stem cell factor (SCT), mast cell growth factor (MCGF) and c-kit ligand. The population of mast cells is not fully homogeneous. Two types of cells are distinguished among them. The first type includes MCT cells (mast cells containing tryptase) whose granules contain only tryptase (located in pulmonary alveoli and intestinal mucosa). The second type consists of MCTC cells (mast cells containing chymase and tryptase) whose granules, apart from tryptase, contain chymase (located in the dermis, intestinal submucosal layer and blood vessels) [6]. The numerical ratio of MCTC to MCT is varied. It depends on the type of tissue. The ratio is different under health and disease conditions. MCTC predominate in the skin, while MCT prevail in lungs and gastrointestinal mucosa. Allergic reactions involve MCT which migrate to the nasal and bronchial epithelium [6].

A mastocyte is typically identified with an immediate hypersensitivity reaction, i.e. a type I reaction according to the Coombs and Gell classification, where it plays a key role [7]. Tryptase is one of many mediators released from mast cells involved in immediate-type reactions. [8, 9]. Other substances stored in granules (preformed [including tryptase]), exhibit a pharmacological effect on blood vessels of the respiratory tract and the respiratory tract itself, resulting in clinical symptoms of hay fever, bronchial asthma, urticaria, or anaphylaxis.

Data presented in the literature also suggests that mast cells are involved in inflammatory processes in the course of other diseases. Attention is drawn to the role of mast cells in autoimmune diseases such as: rheumatoid arthritis, scleroderma or pemphigoid.

The process of releasing substances stored in granules is referred to as “degranulation”. Degranulation of mast cells is an active process of secretion whose essential elements are energy and calcium. A mastocyte can be activated both immunologically and non-immunologically. The former can occur in two ways: in the mechanism dependent and independent of IgE antibodies. Mast cells have membrane receptors for these antibodies. When a mutually complementary antibody joins the membrane receptor and then the mastocyte contacts an allergen, the cell is degranulated [10, 11]. Many non-specific stimuli may, however, release its mediators from the cell. Among them are trauma, high temperature and toxins.

The cytoplasm of a mastocyte contains numerous basophil granules which consist of such substances as histamine, serotonin, glycosaminoglycans, peroxidase, superoxide dismutase, eosinophil chemotactic factors (ECF), neutrophil chemotactic factors (NCF), tryptase, chymase, heparin and numerous cytokines.

The role of cytokines is associated with the initiation of the late allergic response (LAR), or the chronic phase of allergic inflammation (chemotaxis, infiltration and accumulation of inflammatory cells). Clinically, the late allergic response occurs analogically to the early symptoms. It can begin 2–4 hours after the immediate allergic reaction. It peaks after about 8–12 hours and lasts up to 24 hours.

**Tryptase**

Tryptase is an enzyme specific to mast cell granules. The enzyme is present in complexes with heparin and has a high molecular weight compared to histamine (approximately 140 kDa). The genes responsible for tryptase development and synthesis are located on the short arm of chromosome 16. In the human body, depending on the ami-
no acid composition, it is present as: α-tryptase, β-tryptase, γ-tryptase and δ-tryptase. Each of them has subtypes. Table 1 presents the characteristics of each type.

Resistance to protease inhibitors characteristic of tryptase results from an unprecedented ability of this protein to create molecules with a tetramer structure and the active center facing the inside and unreachable to proteins with inhibiting properties [14]. The regulation of tryptase activity due to the specific protection mechanism is not subject to protease-protease inhibitor dependence. The only suggested regulatory mechanism, based on observations in vitro, is the pH-dependent ability of tryptase to bind to the heparin molecule, which facilitates the formation of active tetrameres. At neutral pH, this ability disappears, and tetramers disintegrate. Acidic pH is conducive to forming tetramers [15].

Studies describe the multidirectional effects of the biological activity of tryptase. It causes the disintegration of bronchodilator mediators, which may result in a smooth muscle spasm in the respiratory tract. In the endothelium of blood vessels, their nitric-oxide-dependent vasodilation and increased permeability with the characteristics of hypotension have been observed. Tryptase degrades the vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP). It also exhibits mitogenic activity for fibroblasts and increases expression of the first vascular cellular adhesion molecule (VCAM-1). Tryptase shows activity against plasma coagulation factors (fibrinogen degradation, prekallikrein activation, kinogen distribution, activation of the pro-urokinase-type plasminogen activator (pro-uPA)), whereas the end result is the anticoagulant activity [16]. Tryptase secretion from mast cells depends on the availability of intra- and extracellular calcium and magnesium resources, as well as the presence of phospholipase A2 (PLA2) and phospholipase C (PLC) inhibitors [17, 18].

Determination of tryptase concentration is used as a marker of mast cell activation in inflammatory diseases as the enzyme can be described as typical of mast cells. Available in the pharmacological market, the reference set (UniCAP 100 Phadia, Pharmacia, Uppsala, Sweden) makes it possible to determine the total tryptase concentration. It is a fluorescence immunoassay [19]. Antitryptase reacts with tryptase in the patient serum specimen. After washing, enzyme-labeled antibodies against β-tryptase are added to form a complex. After incubation, unbound enzyme-antitryptase is washed away and the bound complex is incubated with a developing agent. At the end of the reaction the fluorescence is measured, and the degree of fluorescence correlates with the amount of tryptase present.

An increase in serum tryptase levels is observed between 3–6 hours after the onset of an anaphylactic reaction. It returns to normal within 12–14 hours [20]. The practical significance of this is the window of opportunity for testing for systemic anaphylaxis. Tryptase testing can be performed on blood samples obtained 1–6 hours after onset of the reaction. The working party of the Association of Anaesthetists of Great Britain and Ireland on ‘suspected anaphylactic reactions associated with anaesthesia’ advises taking three samples [21]. One immediately after the initial resuscitation, another 1 hour after the start of the reaction at the moment the MCT concentration normally peaks and the last simple 24 hours after the reaction to get a baseline value. The sample taken 1 hour after the start of the reaction is obviously the most important.

<table>
<thead>
<tr>
<th>Type (Typ)</th>
<th>Subtype (Podtyp)</th>
<th>Characteristics (Charakterystyka)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tryptase</td>
<td>α I</td>
<td>– exhibits little biological activity, present in low concentrations in blood serum even in the absence of mast cell degranulation</td>
</tr>
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<td></td>
<td>α II</td>
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</tr>
<tr>
<td>β-tryptase</td>
<td>β 1</td>
<td>– the main form stored in granules of mast cells, released during severe inflammation, such as anaphylactic shock. Absent in serum</td>
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<tr>
<td></td>
<td>β 2</td>
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<td></td>
<td>β 3</td>
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</tr>
<tr>
<td>γ-tryptase</td>
<td>γ I</td>
<td>a protein embedded in the cell membrane of both mast cell granules and its outer membrane, its function is unknown</td>
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<td></td>
<td>γ II</td>
<td></td>
</tr>
<tr>
<td>δ-tryptase</td>
<td>δ I</td>
<td>the type has a significantly shorter chain and different affinity for peptides</td>
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<td></td>
<td>δ II</td>
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</table>
The Application of Tryptase Level Determination

The longer half-life of tryptase in serum makes it a more convenient diagnostic marker than histamine. Histamine can also be released by other cells such as basophils, antigen-presenting cells and bacteria. The optimum time for tryptase determination is between 1–6 hours after the exposure to an allergen (peak secretion between the fifteenth minute and the second hour, with a half-life of 1.5–2.5 hours). It should be remembered, however, that a higher level of tryptase may be observed in diseases and conditions other than anaphylactic allergy. The examples can be wound healing, scar formation involving the mast cell, multiorgan trauma, hypoxia, myocardial infarction, heroin intoxication, mastocytosis. An increase in tryptase levels beyond systemic mastocytosis is also diagnosed in acute myeloid leukemia, myelodysplastic syndromes, hypereosinophilic syndrome related to FIP1L1-PDGFRA mutation, the exogenous administration of SCF (stem cell factor) and end-stage renal insufficiency with increased endogenous SCF levels. Systemic mastocytosis is characterized by mast cell hyperplasia in bone marrow, skin, liver, spleen and gastrointestinal mucosa [22]. A study of tryptase levels in patients with biopsy-proven mastocytosis reported concentrations of total tryptase > 20 ng.mL⁻¹ and ratios of total tryptase to β-tryptase > 20, whereas normal patients had total tryptase levels < 14 ng.mL⁻¹ [23]. The sensitivity of the total mast cell tryptase concentration as a diagnostic test is 83%, with a specificity of > 98%. Total serum tryptase concentrations may be useful in evaluating treatment aimed to reduce mast cell numbers in patients with systemic mastocytosis.

Myelodysplastic syndrome (MDS) is a large group of acquired neoplastic disorders of the bone marrow most common in the elderly and is caused by an abnormal differentiation and maturation of haemopoetic cells. Serum tryptase levels can be used to differentiate the MDS variants. Follow-up studies should clarify whether an elevated serum tryptase concentration in MDS is of prognostic significance.

Serum tryptase determination can be used to evaluate the allergenic activity of drugs taken during anesthesia and the perioperative period. A single tryptase level test can be insufficient, therefore, in order to improve the specificity and sensitivity of determination, a serial test and its comparison with the basic concentration is suggested. It was shown that suxamethonium did not cause the release of this substance from both the basophils of peripheral blood and mast cells of the skin and lungs. On the contrary, after a dose of atracurium and vecuronium – the effect of tryptase release was directly proportional to the dose used, and the dynamics of concentrations stimulated by aminosteroid and benzylisoquinoline drugs was similar. High intersubject variability was observed in atracurium activity in response to mast cell tryptase secretion from the lungs. Among the analyzed drugs, only atracurium resulted in the release of histamine from mast cells obtained from the atria of the heart. Intradermal stimulation of mast cells with aminosteroid and benzylisoquinoline relaxants caused a dose-dependent biochemical effect (tryptase release) and increase in objective (erythema) and subjective (pain, itching) symptoms. In the case of suxamethonium and benzylisoquinolines, the occurrence and intensity of intradermal stimulation subjective symptoms (itching, erythema and pain) corresponded with increasing concentrations of tryptase, which was not observed in relation to aminosteroids [18, 24].

Tryptase was also used in the diagnosis of fatal anaphylactic reactions. It is suggested that an elevated level of tryptase collected post-mortem may persist up to the third day after death from anaphylaxis [25]. Such a determination of serum tryptase in the event of a suspected anaphylactic reaction is useful in most cases of parenteral penetration of allergens. This was proved in post-mortem examinations carried out up to 24 hours after the death of 19 victims. A tryptase level above 10 ng/ml was found in nine out of nine victims of anaphylaxis who were bitten by insects, two of two cases of anaphylaxis after drugs given parenterally, and six of eight cases of food allergy. At the same time, a level below 5 ng.mL⁻¹ was observed in 57 cases of post-mortem serum analysis in persons who died of other reasons than anaphylaxis [25].

Determination of serum tryptase levels was widely used in the diagnosis of mastocytosis. A tryptase level above 25 ng.mL⁻¹ is one of the diagnostic criteria for the disease, although it is not a specific marker of mastocytosis. The diagnosis of this disease is also affected by clinical features and a histopathological picture of the bone marrow.

A number of laboratory methods for determining tryptase levels in bodily fluids have been developed. The radioimmunoenzymatic method is the most useful and the most common method which makes it possible to detect β II-tryptase, specific to allergic processes, at concentrations of less than 1 ng.mL⁻¹, and the range of determinability on the calibration graph is 1–200 ng.mL⁻¹ [11]. It has been shown that in the time interval up to 6 hours after the first symptoms of anaphylaxis, there is no correlation between the time of blood collection and
the recorded tryptase levels [11, 19]. Kinetic parameters and enzyme stability under different external environmental conditions make it possible to collect biological material, and then to conduct quantification of tryptase not only at the time of the onset of allergic symptoms. It is recommended to collect blood for clot in the amount of 5–10 ml, within 1–4 hours from the onset of allergic symptoms [4, 18, 19, 26]. It is recommended to compare the resultant level of tryptase with a control value, obtained at least 24 hours after an anaphylactic reaction. Its elevated level in comparison with the control value is a highly sensitive indicator of anaphylactic reaction in the perioperative period [4].

However, there is a group of patients with clinical symptoms of severe anaphylactic reaction, but their tryptase is maintained at normal levels. They require further diagnostic tests. Namely, an elevated tryptase level does not always correlate with severe anaphylaxis. A negative test result of a tryptase level does not justify a withdrawal from further examination. No increase in tryptase concentration in each case of anaphylaxis is attributed to its local release (e.g. in laryngeal edema) which is not sufficient to increase the total serum concentration, or a greater participation of basophils than mast cells in the pathomechanism of anaphylaxis in a given patient.

Perioperative anaphylactic reactions are a very important anesthesiological problem, because most often they result from the effects of drugs used for general anesthesia. The greatest risk of allergic reactions is associated with the anesthesia induction phase, and skeletal muscle relaxants are one of the allergenic factors. Aminosteroids trigger allergic reactions more often than benzylisoquinolines – constituting about 50% of cases, among them rocuronium (30%), vecuronium (17%) and pancuronium (6%). Among benzylisoquinolines, allergic reactions most often occur after atracurium and mivacurium, 20% and 6% respectively. The fewest allergic reactions are observed after cisatracurium – 0.3% of cases. Depolarizing relaxants (suxamethonium) cause anaphylaxis as often as drugs from the group of non-depolarizing aminosteroids.

In a case of suspected anaphylaxis during anesthesia, it is important to conduct three basic diagnostic tests: biochemical determination, serological determination and skin tests. Tryptase concentration determination is a reliable and effective method of confirming the incidence of anaphylaxis.

The multidirectional effects of drugs used in the perioperative period makes anaphylaxis a very complex problem. This is a very topical issue, and epidemiological data proves the problem is really serious. The severity of an allergic reaction in the final assessment of the suitability of measuring tryptase level as an important element in the diagnosis of anaphylaxis in the perioperative period should undoubtedly be taken into account. However, determination of tryptase levels can not be the only diagnostic tool in perioperative anaphylaxis. A detailed medical history to define the causative agent and clarify the accompanying clinical symptoms should play the primary role. The diagnosis of anaphylaxis is particularly difficult or even impossible in the perioperative period due to the lack of cooperation with the patient (influenced by soporific drugs) and because of the large number of medicines used both for anesthesia and perioperative care. The diagnosis of anaphylaxis in the perioperative period, despite the constant expansion of knowledge, is still a difficult challenge in daily clinical practice. Difficulties in assessing individual risk of anaphylaxis result from a lack of sensitive, widely available diagnostic methods confirming an anaphylaxis episode, and the inability to distinguish between people who are allergic to an allergen and those in whom the exposure to a given factor may cause a life-threatening reaction.

References


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