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Trypsin and Chymotrypsin

MAK Value Documentation – Translation of the German version from 2016

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Trypsin (EC 3.4.21.4) and Chymotrypsin (EC 3.4.21.1)

MAK Value Documentation

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Abstract

Trypsin (EC 3.4.21.4) and Chymotrypsin (EC 3.4.21.1) are proteolytic pancreatic enzymes which are secreted as inactive precursors trypsinogen and chymotrypsinogen, respectively. They have several clinical pharmacological as well as laboratory applications and they are used in protein chemistry, especially in the preparation of insulin. Exposure to enzyme dusts has long been known to cause occupational immediate hypersensitivities of the airways. Also trypsin and chymotrypsin are potential inhalable sensitizers; cases of specific airway sensitization caused by trypsin and chymotrypsin containing products could be shown clearly by several studies. Positive skin prick and challenge tests as well as specific IgE antibodies have been described. Since these results and the clinical symptoms usually matched well, an immunological mechanism of action is suggested. There is no clear evidence of allergic cell-mediated late type eczematous skin reactions. Trypsin and chymotrypsin are designated with "Sa" (for substances causing airway sensitization) but not with "Sh" (for skin-sensitizing substances).

Keywords

trypsin; chymotrypsin; allergenic effects; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Trypsin and Chymotrypsin

Sensitization (2015) **Sa**

Trypsin	9002-07-7
Chymotrypsin	9004-07-3

Trypsin and chymotrypsin are important digestive enzymes that are secreted by the pancreas as the inactive enzyme precursors trypsinogen and chymotrypsinogen. Trypsin activates itself via positive feedback and converts chymotrypsinogen and other inactive enzymes into their active forms. As these enzymes contain serine as the functional amino acid in the active centre, they are classified within the group of serine endopeptidases. Trypsin and chymotrypsin possess a very similar tertiary structure, although they cleave proteins at different positions in the polypeptide chain as a result of different substrate binding sites. In the EC nomenclature for the classification of enzymes, trypsin is listed under the number EC 3.4.21.4 and chymotrypsin under EC 3.4.21.1.

Proteins from food are initially cleaved in the stomach under acidic pH conditions by the enzyme pepsin into peptide fragments. Subsequently, in the small intestine, these are cleaved under alkaline conditions into oligopeptides and amino acids by trypsin and chymotrypsin. For this, the optimum pH range for both enzymes is between pH 7 and pH 8.

Trypsin and chymotrypsin are used in various forms as pharmaceuticals and in research. An example of applied enzyme therapy is the field of wound healing, where the proteinases are used in ointments to avoid necrosis and to improve the absorption of antibiotics. In the treatment of inflammation, trypsin und chymotrypsin, often also in combination with papain, inhibit inflammatory oedema. Furthermore, trypsin is frequently used in cell cultivation laboratories and in protein chemistry. In the pharmaceutical industry, for example, it plays a key role in the production of insulin.

Trypsin and chymotrypsin are usually isolated from porcine or bovine pancreas. That is why these enzymes are frequently obtained as a mixture. Pancreatin, a powdery extract derived from porcine pancreas, more rarely from bovine pancreas, comprises a complex mixture of lipases, amylases and various proteases as the main ingredients. In the meantime, the production of trypsin and chymotrypsin is also possible with the aid of genetically modified microorganisms.

In the early 1950s an American (US) company marketed a medicinal product containing a preparation of 5 mg trypsin/ml sesame oil. This and similar preparations were used for intramuscular injection to reduce local inflammation particularly on eyes and veins and several severe anaphylactic reactions occurred (Criep and Beam

1964). In addition, from the 1960s and 70s onwards, trypsin aerosol inhalation therapy was used, particularly in patients suffering from cystic fibrosis. The protease, which was frequently used as a raw extract in the form of pancreatin, was intended to help liquefy the viscous bronchial mucus of the person affected via mucolysis. Here also cases of sensitization are described, often in parents taking care of their ill children (Ganier and Lieberman 1979; Twarog et al. 1977).

Allergenic Effects

As simultaneous exposure to both trypsin and chymotrypsin often occurs at the various workplaces, the corresponding literature on both enzymes was evaluated together. The literature describes a number of cases of occupational sensitization to pancreatin as the raw extract without testing single enzymes (Hayes and Newman Taylor 1991; Lipkin and Vickers 1987; Shin et al. 2008). Due to the fact that pancreatin also contains other enzymes than trypsin and chymotrypsin, these publications have not been included in the evaluation.

Effects in humans

Sensitizing effects on the skin

Although individual reports of immediate urticarial reactions after contact with trypsin or chymotrypsin are available (for example Johnsen et al. 1997), there are no clinical findings of contact allergic reactions of the delayed type.

Sensitizing effects on the airways

As early as 1957, the case of a 28-year-old laboratory worker was described. In the autumn of 1955 he suffered for the first time from rhinitis and conjunctivitis while weighing chymotrypsin powder, after previously working with the substance for 3 years. The result of a scratch test with 1% chymotrypsin solution was positive, but negative when repeated on the following day. In an intracutaneous test with 0.025 mg chymotrypsin the patient produced a 4+ reaction at the test site, and pruritus occurred in the nose and throat after 15 minutes. After another 15 minutes the patient suffered from a generalized reaction, which required the administration of epinephrine. In a Prausnitz-Küstner test, 3 non-sensitized volunteers were each given injections with 0.1 ml of the patient's serum and 24 hours later with 0.05% chymotrypsin. There was a 3+ skin reaction in 2 of the volunteers (MacLaren and Aladjem 1957).

Two other reports of occupational sensitization to chymotrypsin and trypsin likewise involved laboratory workers. In the first case, rhinitis and conjunctivitis occurred on contact with chymotrypsin in a 29-year-old man about one year after starting work. In a presumably intracutaneous skin test, a pronounced 4+ reaction

was obtained with 1 μg chymotrypsin. Although the patient discontinued working with chymotrypsin, similar symptoms recurred after working for one year with trypsin. In the skin test, the patient produced 3+ and 4+ reactions to 0.1 μg of trypsin and chymotrypsin, and to both enzyme precursors and both enzymes in inactivated form. In a Prausnitz-Küstner test, after transferal of 0.1 ml of the patient's serum to a healthy volunteer the application of the antigens led in all cases to clear skin reactions (Howe et al. 1961). In the same publication, the authors described a further case from the same laboratory. A 21-year-old male who had contact with both trypsin and chymotrypsin suffered from pollinosis, although he denied having allergic symptoms after exposure to the enzymes. In spite of this, he produced a 2+ reaction to trypsin and a borderline positive reaction to chymotrypsin in the skin test (Howe et al. 1961).

A 43-year-old man engaged in obtaining raw extracts of trypsin or chymotrypsin from the pancreas of pigs and cattle, developed a cough and shortness of breath about one year after starting work. Although he noticed a relationship between the symptoms and contact with trypsin and chymotrypsin powder, he continued working for another 6 years before presenting himself at a clinic because of daily attacks of asthma. In the lung function test, the patient was found to have a reduced vital capacity and expiratory flow at 50% of the forced vital capacity. A positive reaction with a wheal of 12 mm diameter was obtained in the scratch test with 1 mg trypsin raw extract/ml. In addition, the result of an intracutaneous test with a 1:200 diluted pharmaceutical preparation of 5 mg trypsin/ml sesame oil was positive, showed a wheal with a diameter of 15 mm. In 50 allergic control persons without evidence of sensitization to trypsin, only one comparable reaction was obtained with the same preparation. A bronchial challenge test performed with a preparation of 10 mg trypsin/ml had to be discontinued after the inhalation of 0.3 ml of this trypsin solution because of coughing and shortness of breath. After inhaling the same trypsin dose, two control persons had no symptoms at all. In this article, the authors drew attention to the potential danger of sensitization to trypsin as a result of the trypsin aerosol inhalation therapy frequently used at the time (Zweiman et al. 1967).

In a study involving 14 persons employed in the industrial extraction of porcine trypsin, 4 workers complained of workplace-related symptoms, of whom 4 reported shortness of breath, 2 coughing and one rhinitis and conjunctivitis. All 4 persons with symptoms reacted in the scratch test to 1 mg trypsin/ml, which had previously been inactivated using a trypsin inhibitor in order to exclude a false positive reaction resulting from enzymatic activity. The results of an identical test with the trypsin inhibitor alone were negative in all cases. The incubation of leukocytes with inactivated trypsin led to the release of histamine in all 4 cases, reaching its maximum with a trypsin concentration of 0.01 μg/ml. The passive transferal of the serum from two persons with symptoms, who reacted in the skin test positive, to a Rhesus monkey, and a subsequent skin test with 12.5 μg trypsin (50 μl of a 0.25 mg/ml trypsin solution) likewise yielded positive results. In a bronchial challenge test in all 3 tested trypsin-sensitized persons, respiratory symptoms of the immediate type occurred at trypsin concentrations of 10 μg/ml, with a reduction in the forced expiratory

volume per second (FEV_1) of 17%, 30% and 32%. All the tests were also carried out with control persons; the results of these tests were negative (Colten et al. 1975).

A 34-year-old male nurse suffered initially from a dry irritating cough without demonstrable infection. Two months later repeated attacks of asthma occurred. While crushing medical preparations, a large oedema developed on one eyelid within a few minutes and asthmatic conditions occurred when handling an ointment containing trypsin. A scratch test with a multienzyme preparation resulted in a pronounced reaction. Using the radio-allergo-sorbent test (RAST), the presence of specific IgE antibodies to porcine trypsin, but not to bovine trypsin, was demonstrated in the serum of the person. In addition, sensitization to an amylase and to pepsin was found. The authors describe a second but non-work-related case of sensitization to trypsin. After treatment of her chronic ulcera cruris with an ointment containing trypsin, the 63-year-old patient developed coughing and dyspnoea within 30 minutes. In the RAST a medium IgE concentration to trypsin and the trypsin preparation was detected in her serum (Hartmann et al. 1984).

Two female employees in a hospital pharmacy aged 26 and 28 years suffered from asthmatic conditions, which they associated with exposure to pancreatin. One of the women suffered additionally from conjunctivitis and eczema. Prick testing with 10^{-7} mg pancreatin/ml yielded positive results in both cases with a wheal 5 to 10 mm in diameter. By means of the RAST, specific IgE antibodies to the pancreatin total extract (19% and 20% IgE binding), but also to porcine trypsin (30% and 26% binding) and bovine trypsin (23% and 19% binding) were demonstrated in the serum of both patients (van Toorenenbergen et al. 1991).

In another study, 10 workers of a pharmaceutical company were examined. All were sensitized to one of the enzymes they were using: trypsin, chymotrypsin, papain, bromelain, amylase or lipase. Of these ten, 5 reported work-related symptoms. In 8 of the 10 sensitized workers, both the prick test with 0.01 to 1 mg trypsin/ml and that with chymotrypsin yielded positive results. Of these eight, 6 were found to have specific IgE antibodies to trypsin in the RAST, 5 of these six also had IgE antibodies to chymotrypsin. All test results were negative in 10 non-exposed control persons. Sensitization to proteolytic enzymes was thus observed more frequently than to the enzymes amylase and lipase (Zentner et al. 1997).

In a retrospective follow-up study, the data of 1064 persons employed in enzyme production were evaluated. Within a period of 3 years, 5% of the workers suffered from asthmatic conditions and 3% from rhinitic symptoms, in some cases also from urticaria in combination with exposure to enzyme dust at the workplace. In 11 of 288 investigated persons (3.8%) using RAST specific IgE antibodies to trypsin were detected (Johnsen et al. 1997).

Animal experiments

There are no valid experimental studies available for sensitization of the airways.

In connection with the intramuscular injection of trypsin formerly carried out to reduce local inflammation, sensitization studies with rabbits and guinea pigs have been described. In both species, after initial intramuscular immunization and repeated intravenous/intracutaneous injections, precipitating antibodies against trypsin were determined. Three of 6 guinea pigs died as the result of anaphylactic shock (Criep and Beam 1964).

Manifesto (sensitization)

Cases of specific hypersensitivity of the airways resulting from exposure to trypsin and chymotrypsin are well confirmed by the numerous studies. These are mostly case reports, which were mainly clearly work-related. The symptoms, skin test results, evidence of specific IgE antibodies and also the result of specific challenge tests usually correlate well. An immunological mechanism of action can thus be considered as proven. There is no clear evidence of allergic cell-mediated late-type eczematous skin reactions. Trypsin and chymotrypsin are designated with "Sa" (for substances causing airway sensitization) but not with "Sh" (for skin-sensitizing substances).

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