Int J Legal Med (2005) 119: 331–334
DOI 10.1007/s00414-005-0524-1

ORIGINAL ARTICLE

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Usefulness of serum mast cell–specific chymase levels for postmortem diagnosis of anaphylaxis

Received: 22 October 2004 / Accepted: 7 January 2005 / Published online: 26 February 2005

Abstract Chymase, a serine protease, is stored mainly in secretory granules of human mast cells. Serum chymase concentration was examined in 8 autopsy cases with anaphylaxis as well as in 104 control cases without anaphylaxis. It was detected in all 8 cases with anaphylaxis (range 3–380 ng/ml, mean 89.8 ng/ml), while it was detected in only 2 of the 104 controls and was below a detectable level (<3 ng/ml) in the other 102. Serum tryptase levels are known to be a diagnostic indicator of anaphylaxis, therefore the relationship between serum chymase and tryptase levels was investigated in the 8 cases of anaphylactic death; a significant positive correlation was found (r=0.826, p=0.011). Furthermore, chymase was shown to be quite stable in serum. These results showed that measurement of serum chymase levels might be an additional tool for postmortem diagnosis of anaphylaxis.

Keywords Anaphylaxis · Chymase · Mast cell · Postmortem diagnosis · Tryptase

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Introduction

Anaphylaxis is an unanticipated immunologically mediated event that occurs after exposure to certain foreign substances in previously sensitized persons. It is initiated by an antigen that binds to specific immunoglobulin E on mast cells. Subsequently, the mast cells degranulate various kinds of mediators from the secretory granules, which leads to anaphylaxis [1]. The diagnosis of anaphylaxis is made primarily by clinical features such as hypotension and from a history of exposure to anaphylactic stimuli [2].

Postmortem diagnosis of anaphylaxis is sometimes difficult and most often done by exclusion. Although edema of the larynx has been observed in some cases of anaphylaxis [3], it is only found in a limited number [4].

Tryptase is a serine protease stored mainly in mast cell granules [5–7]. Since tryptase is released from the granules at the onset of anaphylactic events, serum tryptase levels are considered a reliable indicator of anaphylaxis [8–10]. Furthermore, measurement of serum tryptase levels is known to be useful for postmortem diagnosis of anaphylaxis, because of its long serum half-life compared with other chemical mediators like histamine [11, 12].

Chymase is also a mast cell–derived serine protease, and has been characterized as an angiotensin II–generating enzyme [13–15]. Chymase is used to determine mast cells and thus the vitality of wounds [16]. A method for determining mast cell–specific chymase concentration was recently devel-

Table 1 Serum chymase and tryptase levels in the eight cases of anaphylactic death. *PMI* Postmortem interval

Case	Sex/age	Cause of anaphylaxis	Time until death (h)[a]	PMI (h)	Laryngeal edema	Chymase (ng/ml)	Tryptase (ng/ml)
1	M/65	Antibiotics	3.5	24	+	380	648
2	M/46	Antibiotics	1.5	21	−	88	433
3	M/65	Contrast medium	3.5	18	−	81	182
4	M/70	Antibiotics	1	24	+	78	60
5	M/74	Local anesthetics	1.5	25	−	67	395
6	F/68	Contrast medium	2.5	18	−	14	8
7	M/55	Antibiotics	17	24	−	7	62
8	F/4	Local anesthetics	<1	<48	+	3	16

[a]Interval between the onset of anaphylaxis and death

oped [17]. In the present study, we investigate the possible usefulness of serum chymase levels for postmortem diagnosis of anaphylaxis using this method. We show successful determination of serum chymase levels in serum samples from victims of anaphylactic death with a significant positive correlation to serum tryptase levels. We also demonstrate the usefulness of this method for postmortem diagnosis.

Materials and methods

Quantification of serum chymase and tryptase levels

Blood was collected from the cardiac cavity, centrifuged, and the serum immediately frozen at −20°C until use. Chymase levels were assayed as described previously using a kit supplied by Otsuka Pharmaceutical Co. (Tokushima, Japan) [17]. Sera from 112 adult autopsy cases were analyzed. The causes of death included 8 cases of anaphylaxis, 13 of CO intoxication, 12 of hypothermia, 21 of asphyxia by cervical compression, 28 of myocardial infarction, 18 of cerebral hemorrhage, and 12 of pulmonary thromboembolism. The diagnosis of anaphylaxis was made primarily by clinical features and from the history of exposure to anaphylactic stimuli; it was further supported by the elevation of serum tryptase levels and/or autopsy findings including microscopic evaluations. Tryptase levels were determined using Pharmacia Uni-CAP Tryptase reagents and a Pharmacia Uni-CAP100 analysis device (Pharmacia and Upjohn, Uppsala, Sweden). This study was approved by the ethics committee of Osaka Medical College, Japan.

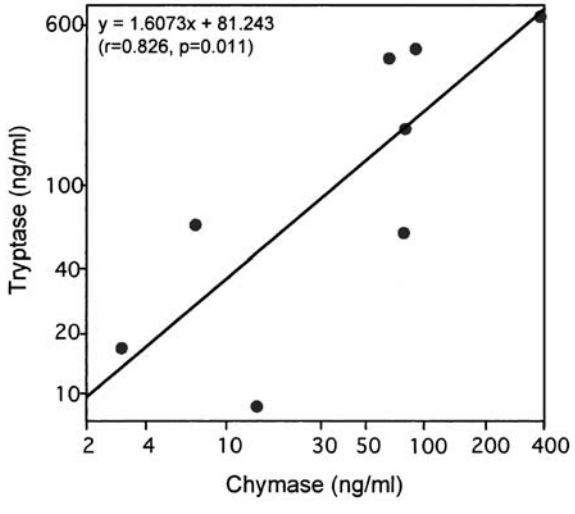

Fig. 1 The relationship between chymase and tryptase levels in serum samples from eight cases of anaphylaxis; a significant positive correlation was found (r=0.826, p=0.011, n=8, Spearman's nonparametric test).

Results

We examined chymase levels in sera from 8 autopsy cases who died of anaphylaxis. Levels of serum chymase concentration were detected successfully in all 8 cases (Table 1). We also investigated the levels in sera from 104 control cases without anaphylaxis. Serum chymase levels were detected in only 2 of the control cases and were below a detectable level (<3 ng/ml) in the remaining 102. Both of the chymase-positive cases in the control group died of myocardial infarction with values of 36 and 30 ng/ml.

Since serum tryptase levels are known to be useful for postmortem diagnosis of anaphylaxis, we examined the re-

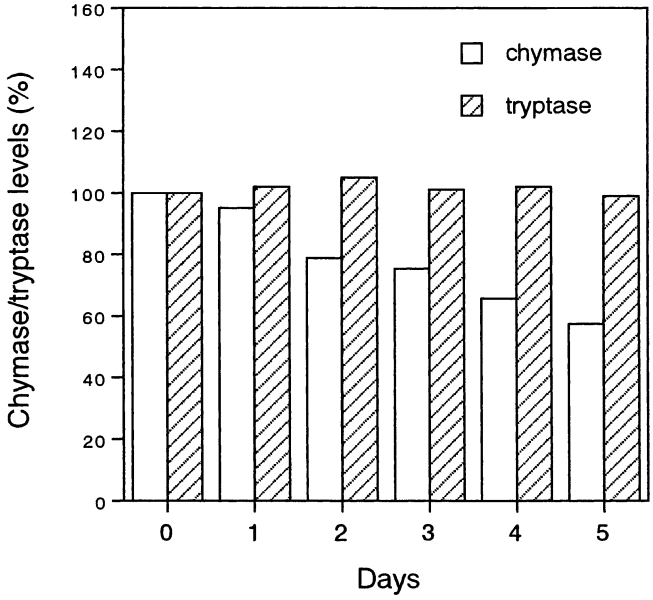

Fig. 2 Effect of incubation time of the serum at 30°C on chymase/tryptase levels. Sera from cases 2 and 3 were examined, and the average level is shown.

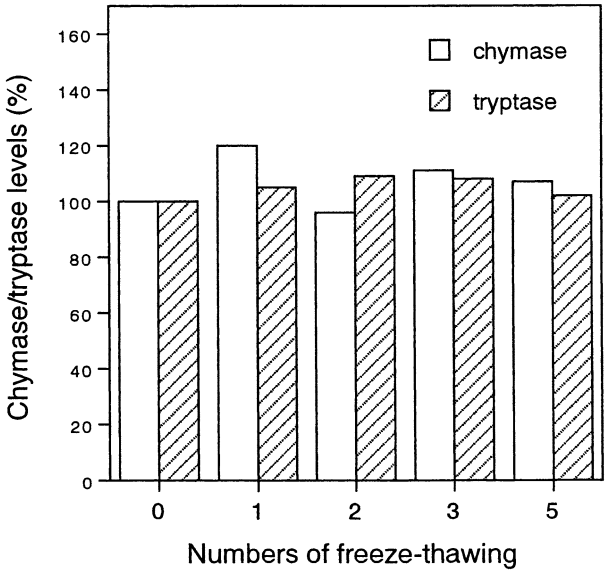

Fig. 3 Effect of repeated freezing and thawing on chymase/tryptase levels. Serum samples from cases 2 and 3 were examined, and the average level is shown.

lationship between chymase and tryptase levels in all eight chymase-positive cases. We found a significant positive correlation between the two (r=0.826, p=0.011) (Fig. 1).

We therefore investigated whether measurements of serum chymase levels are useful for postmortem diagnosis. Firstly, we examined the effect of the time course of serum incubation. Serum chymase levels declined gradually with increasing incubation at 30°C, but almost 60% of the initial level was maintained after 5-day incubation (Fig. 2). Serum tryptase levels did not decline during 5-day incubation. Secondly, we checked the effect of repeated freezing and thawing procedures on chymase levels; neither serum chymase nor tryptase levels declined during these procedures (Fig. 3).

Discussion

In the present study, we measured serum mast cell–specific chymase levels using a recently developed method. Serum chymase levels were detected in only 2 out of 104 cases of nonanaphylactic death. A previous report also failed to detect serum chymase levels in 13 cases of chronic hepatitis and 20 healthy individuals [17]. Taken together, these results suggest that serum chymase levels might be detected in only a limited number of nonanaphylactic-death cases. On the other hand, serum chymase levels were detected in all 8 cases of anaphylaxis. The levels detected varied among the 8 cases examined (Table 1); cases 7 and 8 showed limited elevations of serum chymase with values of 7 and 3 ng/ml, respectively (Table 1). However, in case 7, the level might have been reduced with time (17 h) after onset of the anaphylactic event. In case 8, the serum chymase level might have been low because death occurred immediately at the onset of the anaphylactic event. Intervals of 20 or 30 min after onset of anaphylactic reactions are reportedly necessary for elevation of serum tryptase levels to occur [9, 11].

We also demonstrated a significant positive correlation between serum chymase and tryptase levels in eight anaphylaxis cases (Fig. 1). Considering the usefulness of seum tryptase levels for diagnosis of anaphylaxis [8–12], serum chymase levels might be an additional diagnostic tool.

Of the 104 controls, 2 showed elevated serum chymase concentrations and both died of myocardial infarction. Activated mast cells were previously shown to accumulate at the site of atheromatous rupture in myocardial infarction [18]. In addition, chymase was activated in the pathophysiological state after myocardial infarction [19, 20]. These reports imply that elevation of serum chymase levels in these 2 cases might have resulted from activation of mast cells at the site of infarction. It has also been reported that serum tryptase levels are elevated in a limited number of myocardial infarction cases [21, 22].

Besides in cases of myocardial infarction, serum tryptase levels are also known to be elevated in nonanaphylactic cases such as multiple trauma [21, 22] and heroin intoxication [23], although the precise mechanism is still unknown. Judging from the significant positive correlation between serum chymase and tryptase levels in the present study, it is possible that serum chymase levels might also be elevated in these cases.

There are at least two types of mast cells, those positive for tryptase and chymase, and those positive for tryptase but not immunodetectable chymase. Both types have been shown to have different localizations in tissues [24, 25]. Although a significant positive correlation was found between chymase and tryptase levels, further studies might be necessary to examine the possibility of cases with unassociated values.

In conclusion, measurement of serum mast cell–specific chymase levels might be an additional method for postmortem diagnosis of anaphylaxis.

Acknowledgements This work was supported by a grant-in-aid from the Ministry of Education, Science, Sports, and Culture of Japan to H. Nishio (16590550).

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