

Tryptase as Severity Marker in Drug Provocation Tests

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Key Words

Drug allergy · Drug provocation test · Mast cell degranulation · Tryptase

Abstract

Background: In the absence of objective symptoms, it is difficult to assess an adverse reaction during drug provocation testing. We evaluated the value of serum tryptase levels to distinguish between positive, negative and, even more important, so-called ‘hysterical’ reactions (conversion symptoms). The latter are occasionally observed in drug provocation tests when the patient experiences ambiguous subjective symptoms. **Methods:** In a prospective single-center study, 303 patients underwent 785 drug provocation tests. Blood was taken for tryptase measurement on each test day before and after drug challenge, and the changes in serum tryptase levels in patients with no reactions were compared with those who experienced immediate reactions to a drug. **Results:** Thirty-four of 785 drug provocations were clinically judged as being positive. Despite objective symptoms, median serum tryptase values in the afternoon were even lower than baseline levels. However, this decrease was not statistically significant. In the 751 patients suffering no objective reactions, the median values of post-testing tryptase values were statistically significantly decreased as compared with pretesting values. **Conclusions:** The measurement of serum tryptase levels does not ap-

pear to be helpful to differentiate mild allergic or nonallergic reactions from ‘hysterical’ ones. The milder decrease in the group with objective drug reactions might indicate slight mast cell activation in some patients. More severe clinical drug reactions led to stronger mast cell degranulation. Mild reactions did not increase the tryptase levels consistently.

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Introduction

Adverse drug reactions are a common phenomenon [1]. A number of in vitro and in vivo tests are used to diagnose such reactions. Drug provocation testing is the best diagnostic tool despite its limitations [2]. However, the assessment of adverse reactions to drugs during provocation testing is very difficult in the absence of objective clinical signs. The subjective symptoms resulting from the patient’s anxiety and past experiences must also be taken into account [3]. Serum tryptase has been suggested as a marker in cases of anaphylactic or anaphylactoid reactions [4–8], but mostly, the described reactions had been severe.

Tryptase is a neutral protease stored in its mature form in the secretory granules of mast cells and basophils, with levels in mast cells being 250- to 850-fold higher than those in basophils [9]. Tryptase is released after crosslinkage of immunoglobulin E receptors by allergens or other

degranulating agents [10, 11]. This degranulation of mast cells can be mediated either immunologically or nonimmunologically. Both mechanisms play a role in adverse drug reactions [1]. Mast cells exert important functions in allergic inflammatory processes [12], and tryptase levels correlate well with mast cell activation. Our aim was to determine the importance of serum tryptase levels as a tool to objectify mild drug test reactions, in particular for the discrimination of true positive (allergic or nonallergic) from true negative reactions, even if these are accompanied by subjective conversion symptoms. A large number of patients was routinely tested so that a statistically satisfactory answer could be expected.

Materials and Methods

In a prospective study design, between July 1999 and December 2001, 303 consecutive patients with a suspected history of drug hypersensitivity reactions to different drugs were referred for a controlled drug provocation test after providing signed consent. The test was conducted in an institution with extensive experience in this field. Patients with suspected drug intolerance are routinely sent to this department, which is the only one of its kind in southern Austria (approximately 2 million inhabitants). The pretest symptoms reported by 232 patients were localized or generalized pruritus, erythema, urticaria, rhinoconjunctivitis and, in 71 patients accompanied by changes in arterial pressure, dyspnea and gastrointestinal complaints. The time interval from the drug reaction to the provocation test ranged from 3 to 28 months (median 10 months). In patients with suspected allergy to betalactamic drugs, specific serum immunoglobulin E (CAP RAST FEIA, Pharmacia, Sweden) had been measured before admission. Only patients with negative CAP RAST to penicilloyl G and V, amoxicillin and ampicillin were tested. After admission to the inpatient unit, the patients' medical history was recorded and a clinical examination performed. In a second step, skin tests were done. We performed prick tests and intradermal tests with penicillin (penicilloyl polylysine, minor determinant mixture), acetylsalicylic acid, acetaminophen (paracetamol) and local anesthetics. Additional skin testing with amoxicillin and ampicillin was introduced into our standard procedure in 2003, the testing of other betalactamic drugs as proposed by Torres et al. [13] in 2004. Diclofenac was applied epicutaneously (5% in petrolatum and 5% in water). Reading was done 30 min after application. Skin testing was followed by provocation tests (for the sequence of increasing drug dosage and maximum daily drug dosage, see table 1). All tests were performed according to international criteria [2]. The majority of these were oral provocation tests; a few parenteral provocations (intravenous or subcutaneous) were conducted. Eighty-eight tests (11.2%) were performed with the culprit drugs, 697 tests (89.8%) with alternative ones. Blood samples for determination of serum tryptase levels were obtained from every patient on each test day in the morning before starting the test procedure. In the case of no adverse reaction, a blood sample was taken 2 h after the last drug exposure. In cases of adverse reactions, blood was taken 2 h after the onset of symptoms. During the period of exposure, the patients were al-

Table 1. Challenge workup for drugs provoking positive test reactions

Drug	Sequence of increasing DD					Maximum daily DD
AMOX, mg	500	1,000	1,500			3,000
ACET, mg	10	50	100	250	500	910
ASS, mg	10	30	100	250	500	890
DIC, mg	25	50				75
LID 2%, ml	0.1	0.5	1.5			2.1
MEF, mg	10	50	100	250	500	910
OFL, mg	100	200				300
PEN, mg	500	1,000	1,500			3,000

DD = Drug dosage; AMOX = amoxicillin; ACET = acetaminophen (paracetamol); ASS = acetylsalicylic acid; DIC = diclofenac; LID = lidocaine; MEF = mefenamic acid; OFL = ofloxacin; PEN = phenoxymethylpenicillin.

lowed to drink, but not ingest food. Intravenous fluids were not administered, with the exception of a few milliliters for drug testing. The serum tryptase levels from 785 test days were analyzed. This total number resulted from provocation tests with different drugs depending on the patient's history. The tests were performed on consecutive days. Blood samples were centrifuged and stored at -20°C . Sample processing was done with the UniCAP tryptase fluoroimmunoassay (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Provocations were considered to be positive when objective symptoms occurred, which were as follows: erythema, urticaria, rhinitis, conjunctivitis, dizziness, dyspnea and changes of blood pressure (>20 mm Hg). Placebos were administered to detect conversion symptoms when the reaction had not been unequivocal. The patients were divided into two groups: the first consisted of those with positive drug provocation tests, while the second had no objective reactions. In each group, differences between serum tryptase values prior to and after drug challenge were analyzed.

The test for normality (Kolmogorov-Smirnov) failed ($p < 0.001$). Statistical analysis of the median serum tryptase values was performed using Wilcoxon's signed rank test. The assigned p value for statistical significance was <0.05 .

Results

Thirty-four of 785 drug provocations were confirmed to be positive, i.e. reactions of the immediate type, indicative of mast cell involvement (table 2). Twelve reactions to acetylsalicylic acid, 7 to penicillin, 6 to mefenamic acid, 4 to diclofenac, 2 to amoxicillin, and 1 each to paracetamol (acetaminophen), ofloxacin (in all cases oral challenges) and lidocaine (subcutaneous challenge) were seen. Seven hundred and fifty-one provocation tests were

Table 2. Serum tryptase levels in patients with a positive drug challenge

Age	Sex	Drug	I	II	III	IV
16	M	ASS	6.11	12.4	102.9	urticae, head, trunk
61	F	DIC	6.04	9.24	53	urticae, generalized; angioedema, lips
31	F	MEF	2.56	3.59	40.2	angioedema, periorbital
22	M	ASS	1.33	1.64	23.3	urticae, head, trunk
16	M	AMOX	4.56	5.58	22.4	urticae, generalized; angioedema, hands, feet
32	F	MEF	5.87	6.63	12.9	urticae, generalized; angioedema, periorbital
53	M	ASS	6.71	7.3	8.8	urticae, trunk, arms
48	F	MEF	5.51	5.69	3.3	urticae, generalized; vertigo; hypertension
52	M	ASS	5.18	5.25	1.4	angioedema, periorbital right; conjunctivitis
28	F	PEN	4.85	4.89	0.8	urticae, trunk
24	F	PEN	3.8	3.82	0.5	urticae, arms
65	F	ASS	11.4	11.4	0	angioedema, hands, feet, tongue
21	M	OFL	9.12	9.12	0	angioedema, hands, lips; dyspnoe; cough
29	F	ASS	2.11	2.11	0	erythema, hands, feet
51	M	PEN	7.69	7.52	-2.2	urticae, trunk, neck, left thigh
57	M	ASS	6.35	6.1	-3.4	urticae, legs, back
27	M	DIC	6.94	6.67	-3.9	urticae, generalized; tightness of the chest
44	M	MEF	3.23	3.07	-5	urticae, thighs
48	M	MEF	5.52	5.15	-6.7	urticae, legs
54	F	ASS	5.17	4.77	-7.7	urticae, neck, trunk
21	F	AMOX	4.35	4.01	-7.8	urticae, legs, arms, trunk
27	M	PEN	6.01	5.48	-8.8	urticae, trunk, arms
57	F	ASS	7.61	6.8	-10.6	urticae, back, legs
39	M	MEF	9.92	8.64	-12.9	urticae, neck, head
58	M	PEN	7.78	6.7	-13.9	urticae, trunk; erythema, plantar
41	F	DIC	5.83	4.86	-16.6	urticae, generalized
23	F	PEN	3.26	2.7	-17.2	urticae, arms
21	M	LID	4.34	3.46	-20.3	urticae, arm
21	F	DIC	4.79	3.56	-25.7	angioedema, periorbital
38	M	ASS	6.49	4.79	-26.2	urticae, head, neck; tightness of the chest
38	M	PEN	1.37	1	-27	erythema, trunk, head; conjunctivitis; vertigo
20	M	ACET	3.55	2.32	-34.6	urticae, hands, arms
21	F	ASS	8.5	3.35	-60.6	urticae, arms; angioedema, periorbital
46	F	ASS	8.06	2.26	-72	erythema, trunk, head; tightness of the chest

The bold lines divide patients with increased postexposure values from those with a decrease or increase of less than 5% in the comparison between morning and postchallenge values, and those with decreased afternoon values

I = Tryptase before challenge ($\mu\text{g/l}$); II = tryptase after challenge ($\mu\text{g/l}$); III = in-/decrease ($\pm\%$); IV = test reaction; ASS = acetylsalicylic acid; DIC = diclofenac; MEF = mefenamic acid; AMOX = amoxicillin; PEN = phenoxymethylpenicillin; OFL = ofloxacin; LID = lidocaine; ACET = acetaminophen (paracetamol).

classified as being negative (control group). The difference between morning and afternoon values in serum tryptase was statistically significant ($p < 0.001$) in the control group (fig. 1); the values were reduced in the afternoon compared with the morning. In the group with positive test reactions, the median afternoon measurements were also lower than those in the morning, but the difference was not statistically significant ($p = 0.071$) (fig. 2).

Serum tryptase levels increased after positive provocation tests in 7 cases only. One patient experienced an increase of more than 100% (6.11 vs. 12.4 $\mu\text{g/l}$, 102.9%); a second patient had an increase of 53%. These 2 patients developed generalized urticaria, but no orthostatic changes or other systemic signs. In the remaining 5 cases, the increase ranged from 8.8 to 40.2% (table 2). In 11 positive test reactions, no change in tryptase levels (a decrease or increase of less than 5% between pre- and postprovoca-

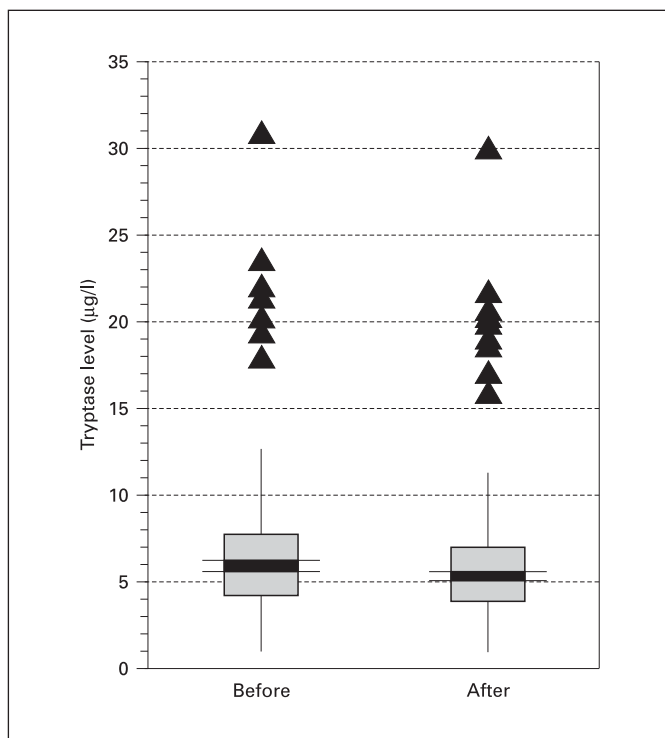


Fig. 1. Tryptase levels in the control group (751 negative provocation tests).

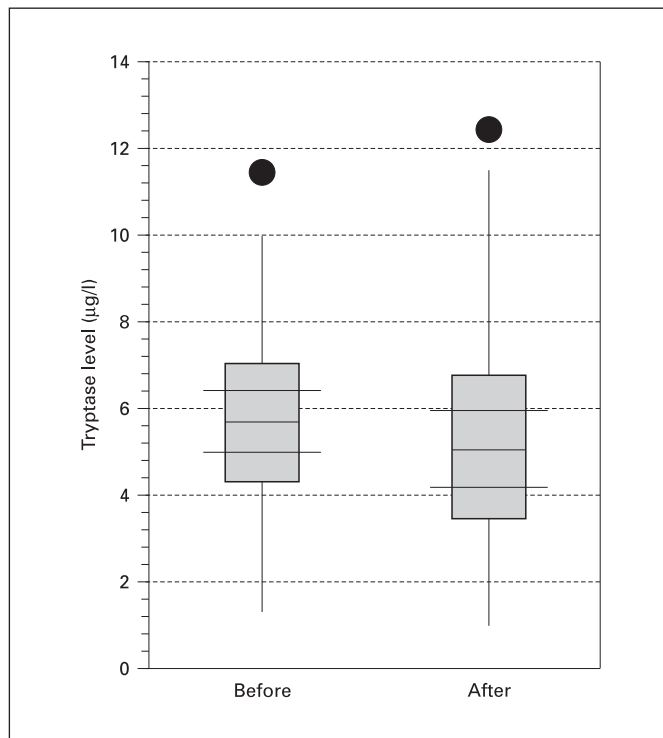


Fig. 2. Tryptase levels in 34 patients with positive drug provocation tests.

tion values) was found. In fact, serum tryptase values were reduced in 16 positive test procedures (table 2).

Seven hundred and fifty-one drug provocation tests were considered to be negative. In 104 of these provocations, the afternoon (postprovocation) tryptase levels were higher than those before testing; the mean increase was 12.7%, whereas the mean decrease of the other 647 negative tests was 12.6%.

Discussion

Reliable laboratory and skin testing procedures for the confirmation of a clinically suspected sensitization are only available for a small number of drugs [14]. Drug provocation testing is frequently required to prove or disprove sensitization to a certain drug. However, provocation tests are potentially dangerous and often associated with subjective symptoms that need further substantiation [2]. A test to measure serum tryptase levels has recently become available. The pathophysiologic and clinical usefulness of serum tryptase determination was confirmed in anaphylactic, allergic and nonallergic, reactions

[4–8]. Tryptase has been measured in several body fluids: nasal lavage fluid [15], tears [16, 17], bronchoalveolar lavage [18] and intra-articular fluid [19]. Postmortem measurement of serum tryptase revealed altered levels in sudden infant death syndrome [20] and in a small number of unexpected deaths, possibly cases of clinically unrecognized fatal anaphylaxis [21].

Increased serum tryptase levels have been reported in drug allergy reactions. It was suggested that the measurement of serum tryptase could be helpful to diagnose adverse drug reactions that are accompanied by mast cell degranulation. Detectable increases have been reported after controlled drug challenges [22, 23]. However, recently, clinicians were advised to be cautious when using serum tryptase to refute or support a diagnosis of anaphylaxis [24]. Some authors observed a remarkable increase in serum tryptase after drug provocation in cases of anaphylaxis, yet doubt the usefulness of serum tryptase measurement in milder reactions [25].

Our observations confirmed this thesis. The most severe symptoms in patients with positive provocation tests were mild erythema, urticaria or mild mucocutaneous symptoms such as sneezing and rhinorrhea. This was be-

cause rescue medication had to be started immediately after the onset of objective symptoms. In all cases, the intravenous administration of antihistamines and corticosteroids was sufficient to prevent a progression to severe reactions. Thus, severe respiratory and gastrointestinal symptoms, hypotension and loss of consciousness could be avoided. Adrenaline was not necessary. Rescue medication reduced clinical symptoms, as an inhibition of mediator release from mast cells is unlikely. In vitro studies have demonstrated that incubation for 24 h with the potent synthetic glucocorticoid dexamethasone does not inhibit mediator release from human mast cells derived from the lung, intestine or skin [26]. Additionally, in vivo administration of glucocorticoids for periods of 48 h or less fails to reduce the magnitude of early-phase allergic responses, indicating that mast cell degranulation is not inhibited [27].

Both the positive and the negative challenge group experienced a reduction in median serum tryptase levels between the morning and the afternoon, i.e. postprovocation measurement. In the numerically larger group with negative provocation tests, this difference was statistically significant ($p < 0.001$), but not so in the positive provocation group ($p < 0.071$). We observed a circadian rhythm in serum tryptase levels although our study was not designed to detect diurnal tryptase variations as the primary endpoint. The small change in tryptase concentration might have been due to a dilutional effect, because our patients had been allowed to drink between blood collection in the morning and the afternoon. The reason for the absence of a statistically significant decrease between morning and afternoon values in the group with positive challenges is not clear. The mild clinical reactions to the administered drugs may be accompanied by the activation of mast cells. The resulting tryptase release is obviously not strong enough to cause a measurable increase in median tryptase values after positive challenge compared with baseline values, but may suffice for a lesser decrease in the afternoon values; the difference may have been simply due to the smaller number of subjects. On the other hand, a mild increase in serum tryptase was registered in some persons in the negative challenge group as well. The measurement of tryptase levels 2 h after the onset of an allergic reaction as proposed by some authors is a practicable procedure in clinical routine [8, 28]. Ordoqui et al. [25] suggest serial blood extractions for optimum results. However, this procedure cannot be applied under routine conditions and would probably not have altered the outcome of the present study.

The rate of 4.3% (34 out of 785) of positive provocation tests seems to be surprising. It is a result of our philosophy not to expose patients to danger but to look for safe alternative drugs. Our patients were thus seldom (88 out of 303 patients) exposed to the culprit drugs.

The purpose of the study was to evaluate the discriminative ability of serum tryptase levels to identify a positive drug provocation test in a large routine test population. This ability was not given. In our study population, drug challenge might result in a very slight mast cell degranulation and a mild increase in serum tryptase levels in some patients. This might provoke a statistically lesser decrease in afternoon levels as compared with baseline in blood samples taken in the morning, even if the absence of a statistically significant decrease in this group might have been due to the smaller number of subjects. Further studies will be required to assess the clinical relevance of this observation. The course of tryptase levels in a single case is not constant, as mild reactions are seen even in the safe environment of a hospital. Therefore, the measurement of serum tryptase levels in a single individual is not helpful. One limitation of our study is that severe adverse reactions after provocation did not occur. These reactions might lead to a substantial increase in tryptase levels. However, in these cases, the clinical signs would be highly objective. The measurement of serum tryptase is thus not useful to differentiate mild allergic or nonallergic reactions from the 'hysterical' ones (conversion symptoms) as occasionally seen in drug provocation tests.

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