

Short communication

Serum tryptase levels in adverse drug reactions

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We evaluated the usefulness of individual tryptase levels and variations after adverse drug reactions in 64 patients. Our aim was to find a tool for the diagnosis of drug allergy. Thirty-seven subjects were confirmed to have drug allergy, 12 had nonsteroidal anti-inflammatory drug (NSAID) reactions, five had negative controlled drug challenges (NAAR), and 10 had symptoms after placebo intake (PLA). Serum tryptase levels greatly increased after anaphylactic shocks (2242%) and anaphylaxis (710.5%). Patients with allergic urticaria and those with idiosyncratic responses to acetylsalicylic acid (ASA) exhibited a small increase in serum tryptase (49.5% and 38.2%, respectively). In the other two groups (NAAR and PLA), no variation in this serum protease was observed. The time of appearance of the serum tryptase peak differed considerably among patients with similar clinical reactions (from 30 min to 6 h) and was independent of the latent period, severity of symptoms, or the amount of tryptase released. We conclude that serum tryptase determinations are helpful in the diagnosis of anaphylactic shock and anaphylaxis, but serial measurements may be needed to confirm mast-cell participation in milder reactions.

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Adverse drug reactions are very common, but the mechanisms involved are usually unknown. When drug allergy causes the reaction, drug avoidance is mandatory and desensitization may be required in special cases. Diagnosis of drug allergy is difficult by the procedures currently available. Skin tests have been shown to be useful for only a few drugs (1), specific serum IgE tests are not sensitive enough (2), histamine basophil release has not yet been standardized, and *in vivo* controlled challenge may be dangerous. Therefore, any *in vitro* test that may help to elucidate drug allergy physiopathology should be considered.

Mast cells play an important role in the allergic inflammatory process (3) and may be target cells in an allergic drug reaction. The high and exclusive tryptase level found in these cells (≥ 10 pg/cell) makes this protease a good marker of mast-cell activation (4–7). Its longer life in blood, compared to histamine, and the current availability of a commercial radioimmunoassay for *in vitro* quanti-

fication (8) makes this a useful tool to study the physiopathology of allergic drug reactions. In this study, we evaluated the utility of changes in serum tryptase levels during adverse drug reactions.

Material and methods

Sixty-four patients from the allergology section and from the emergency unit of "Gregorio Marañón" University General Hospital who had suffered different cutaneous or systemic adverse reactions to drug administration were studied. Diagnosis was established by an allergologic study within a 3-month period after the reaction. Clinical records were examined, and cutaneous tests, specific serum IgE determinations (CAP Pharmacia, Uppsala, Sweden), and/or systemic challenges were performed.

Thirty-seven patients were confirmed to have drug allergy (positive cutaneous test and/or specific IgE, and/or positive controlled drug challenge):

seven anaphylactic shocks (AS), including patients who suffered cutaneous, digestive, and/or respiratory symptoms with hypotension with or without loss of consciousness; 13 anaphylactic reactions (ANA) with similar symptoms but normal arterial pressure values; and 17 cases of urticaria-angioedema (U-AE).

Drug allergy was excluded in 27 patients. Twelve subjects had developed nonsteroidal anti-inflammatory drug (NSAID) idiosyncrasy (IDI), and five suffered nausea, paresthesia, dizziness, pruritus, or erythema and had a subsequent negative controlled drug challenge. This latter group was designated the "nonallergic adverse reaction" (NAAR) group. Ten patients developed different symptoms upon placebo administration (PLA group), and basal tryptase was also obtained in six healthy subjects for control purposes.

The agents implicated were as follows: antibiotics ($n=26$), NSAID ($n=24$), pollen grains ($n=1$), fluorescein ($n=1$), oxybutynin ($n=1$), ergo-
tein ($n=1$), and placebo ($n=10$).

Blood extractions were obtained 2 h after the onset of symptoms (postreaction level [PRL]) and 7 days later (basal level [BL]). Serial blood extractions were also determined in seven patients who developed AS or anaphylaxis. Sera were separated and stored at -20°C for later use. Tryptase levels were measured by radioimmunoassay (Tryptase RIACTTM Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. Briefly, 50 μl of serum was incubated with I^{125} -labeled monoclonal antitryptase in an antitryptase-bound tube for 18 h at room temperature. After washing, radioactivity was measured in a gamma counter. The detection limit of the assay is 0.5 U/l, defined as the concentration indicated by the counts three standard deviations above that of a zero sample. Coefficients of variation within and between assays varied from 1.7 to 2.8 U/l (manufacturer's protocol).

Serum tryptase increase was expressed for each reaction as $[(\text{PRL} - \text{BL})/\text{BL}] \times 100$ and represented as $\Delta\%$.

Statistics

Results were listed as a mean $\pm$ standard error of the mean. Mean group values within groups were compared with the Wilcoxon test. For statistical significance, the assigned P value was <0.05 .

Results

Serum tryptase variations

Serum tryptase levels increased in patients who suffered allergic and nonsteroidal idiosyncratic

reactions. The highest increase was observed in the more severe clinical manifestations: the AS group had 2242% (PRL: 17.62 ± 6.8 U/l, $P < 0.005$), followed by the ANA group at 710.5% (PRL: 7.17 ± 4.9 U/l, $P < 0.005$). Urticaria and idiosyncratic reactions evoked a minimal tryptase increase (49.5% and 38.2%, respectively) (Table 1).

Patients who suffered a reaction upon placebo challenge or subjects of the NAAR group did not experience any increase in tryptase level. Mean basal levels did not differ among groups (Fig. 1).

Time of the tryptase peak value

The time of the peak value of serum tryptase, named as postreaction maximum level (PR max. L), differed widely in the various anaphylactic reactions (Fig. 2). The peak was obtained in the first 30 min in two cases of AS induced by oral erythromycin (PR max L: 53 U/l) and oral cotrimoxazole (PR max. L: 4.09 U/l), and in two cases of anaphylaxis caused by intravenous fluorescein (PR max. L: 66.2 U/l) and oral dipirone (PR max. L: 9.05 U/l). The highest level was detected after 2 h in a patient who developed AS with oral amoxicillin (5.87 U/l). Tryptase peaked 3–4 h after onset of symptoms in AS induced by oral amoxicillin (PR max. L: 27.54–27.38 U/l) and 6 h in another AS caused by oral amoxicillin (PR max. L: 20.7 U/l). Serum tryptase decreased to BL by 24 h in all the patients.

The time of occurrence of the serum tryptase peak level was not related to the severity of symptoms, the latent period of symptoms, or the amount of protease released (Table 2).

Discussion

Tryptase is a useful marker to study the presence and mechanism of mast-cell activation in various diseases and reactions (9). Quantification of

Table 1. Mean tryptase variations during drug reactions. Serum tryptase increase ($\Delta\%$), PRL (mean serum tryptase 2 h after onset of symptoms, postreaction level [standard error of mean, SEM]), BL (mean serum tryptase 7 days after reaction, basal level [SEM]), AS (anaphylactic shock), ANA (anaphylaxis), U-AE (urticaria-angioedema), IDI (idiosyncrasy reactions to NSAIDs), NAAR (nonallergic adverse reactions), PLA (placebo challenges)

Tryptase mean values (U/l)	n	$\Delta\%$	PRL	BL	P value
AS	7	2242	17.62 (6.8)	0.87 (0.06)	0.001
ANA	13	710.5	7.17 (4.9)	0.84 (0.05)	0.001
U-AE	17	49.5	1.1 (0.13)	0.75 (0.04)	<0.01
IDI	12	38.2	0.97 (0.15)	0.70 (0.02)	<0.05
NAAR	5	11	0.93 (0.07)	0.92 (0.2)	NS
PLA	10	6.19	0.94 (0.08)	0.88 (0.06)	NS

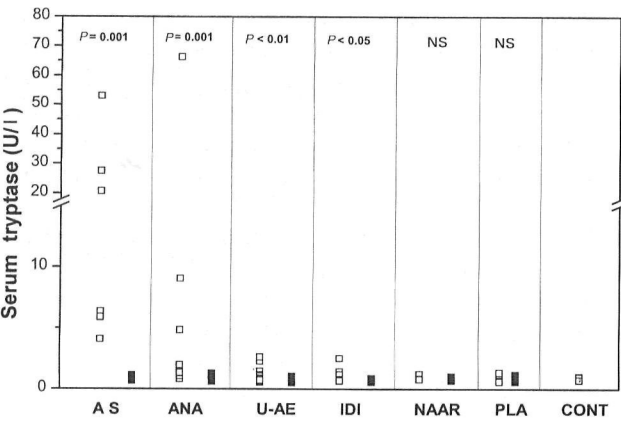


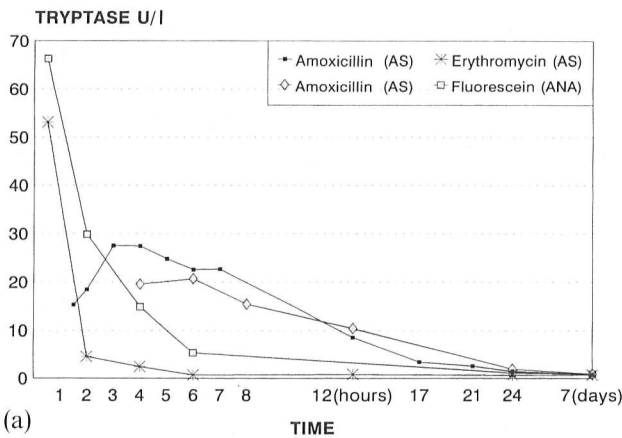
Fig. 1. Patients distributed by groups: AS (anaphylactic shock), ANA (anaphylaxis), U-AE (urticaria-angioedema), IDI (idiosyncrasy to ASA), NAAR (nonallergic adverse reactions), PLA (placebo), CONT (control). Open squares (left) represent postreaction tryptase levels (PRL); closed squares represent basal tryptase levels (BL).

inflammatory markers in allergic reactions induced by drugs is helpful in the identification of any cell type involved and improves insight into the different pathogenic mechanisms (10, 11).

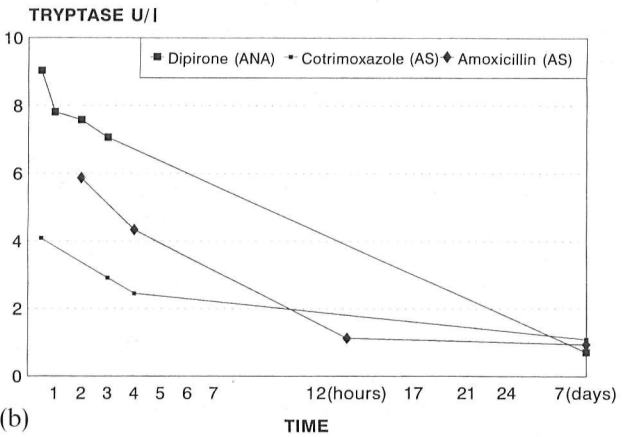
Tryptase levels have been detected in various corporal fluids: tears (12, 13), nasal exudation (14, 15), intra-articular liquid (16), and serum (17–20). It has been suggested that the measurement of serum tryptase levels after adverse drug reactions could be useful for the diagnosis of allergic reactions. We have observed that this protease is present in increased levels in patients with allergic reactions (AS, ANA, and U-AE) caused by drugs (18, 21). Other authors have reported increases in tryptase, histamine, and eosinophil cationic protein in allergic reactions, especially anaphylaxis (17, 19).

The increase in tryptase that we observed in allergic reactions was proportional to the severity of the reaction (AS or anaphylaxis), but inter-individual variability was high. In these groups, measurement of this protease is indeed useful for diagnosis. However, allergic drug urticaria and NSAID idiosyncrasy evoked a minimal increase in tryptase levels which was not sufficient to validate this test for routine diagnosis. In our experience, postreaction tryptase levels, taken 2 h after the onset of symptoms in the last two groups, are very difficult to interpret because PRL values are too close to basal and detectable levels (sensitivity) of this assay.

Although the highest increases were found in the severer syndromes, there was a great variation of tryptase levels in patients with AS or anaphylaxis of similar characteristics (Fig. 2). Probably, other factors such as distribution of allergen, sensitized mast cells, and target tissue responsiveness, not



(a)



(b)

Fig. 2a and b. Serial tryptase values after five anaphylactic shocks and two cases of anaphylaxis.

included in the study and coresponsible for the clinical symptoms, are modifying the mediators' responses.

Determination of the release of this protease is difficult to assess because the time of the peak level and the kinetics of the release differ greatly among patients. The 2-h period, proposed by some authors as the optimal time for blood extractions (17, 19), may not always be suitable. A sole measurement is clearly not sufficient to evaluate mast-cell participation when the increase is small.

The mode of drug administration, latent period of symptoms, amount of tryptase released, or the severity of the reaction do not seem by themselves to correlate in our patients with the time of the peak value. A great interindividual variation was observed (from less than 30 min to more than 5 h). Knowledge of the factors that determine the time of occurrence of the peak value will improve the assay. So far, serial blood extractions are needed for optimum results. A new tryptase assay has been reported that detects active and inactive tryptase (22). This kit should be evaluated in mild allergic reactions.

Table 2. Time of peak tryptase value in relation to clinical manifestations (CM), latent period of symptoms (LP), postreaction maximum level (PR max. L), drug type, and mode of administration (MA)

Time of peak value	CM	LP	PR max. L	Drug	MA
<30 min	ANA	35'	66.21	Fluorescein	I.V.
<30 min	AS	5'	53.02	Erythromycin	Oral
<30 min	AS	10'	4.09	Cotrimoxazole	Oral
<30 min	ANA	2'	9.05	Dipirone	Oral
<2 h	AS	30'	5.87	Amoxicillin	Oral
3.5 h	AS	1'	27.5	Amoxicillin	Oral
6 h	AS	90'	20.07	Amoxicillin	Oral

We looked for increased tryptase levels in patients with idiosyncratic reactions to NSAIDs. Other authors have already reported some detectable increases after controlled challenge procedures (20, 23). The increase in our patients with mainly urticarial or light nasal and ocular symptoms after drug intake was small but suggests mast-cell participation. Possibly in severer reactions, such as bronchospasm, the increase in tryptase would be more evident.

At present, diagnosis of drug allergy is still based on *in vivo* tests that entail some degree of risk. The appearance of new helpful *in vitro* assays for diagnosis is an important and encouraging goal.

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