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Tryptase Levels Are Not Increased during Vancomycin-induced Anaphylactoid Reactions

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Background: **Anaphylaxis, mediated by immunoglobulin E, may be clinically indistinguishable but is mechanistically different than chemically mediated anaphylactoid reactions induced by drugs such as morphine, curare, and vancomycin. A test to distinguish anaphylactic from anaphylactoid reactions would clarify therapeutic and medicolegal issues. Tryptase levels identify anaphylactic reactions but have not been evaluated *in vivo* during anaphylactoid reactions. A prospective, randomized, double-blinded, placebo-controlled trial of antihistamine chemoprophylaxis for rapid vancomycin infusion was performed, and plasma tryptase was measured using a new immunoassay. Histamine release was established by measurement of plasma histamine and the ability of prophylactic H_1 and H_2 antagonists to prevent common histamine-associated side effects. Tryptase levels were compared with histamine levels and clinical symptoms.**

Methods: **Before elective arthroplasty, 40 patients received vancomycin infusion (1 g over 10 min) and pretreatment with either antihistamines (1 mg/kg diphenhydramine and 4 mg/kg cimetidine) or placebo. Changes in tryptase (at peak histamine and 10 min after vancomycin infusion), histamine levels, and histamine-mediated symptoms were assessed using Fisher's exact test, the Student's *t* test, or the paired *t* test, as appropriate. Logistic regression models were used to quantify the association of clinical symptoms with antihistamine treatment and serum levels.**

Results: **Plasma tryptase levels were unchanged (99% CI, −0.5 to 1.6) independent of increased histamine levels, antihistamine pretreatment, clinical symptoms, or all of these. Histamine levels >1 ng/ml were significantly associated with hypotension, moderate-to-severe rash, and stopped infusion. Antihistamine pretreatment significantly decreased the incidence and severity of the reactions.**

Conclusion: **Plasma tryptase levels were not significantly elevated in confirmed anaphylactoid reactions, so they can be used to distinguish chemical from immunologic reactions. (Key words: Anaphylaxis; antihistamines; histamine; red man syndrome.)**

ANAPHYLAXIS is the unforeseen yet life-threatening reaction to agents such as latex and anesthetics. This immunologic disorder occurs in previously sensitized persons who generate immunoglobulin E antibodies, which trigger mast cell and basophil degranulation and result in the co-release of histamine and tryptase.[1] Chemically mediated anaphylactoid reactions appear clinically indistinguishable from anaphylaxis and do not involve immunoglobulin E sensitization. Both anaphylactic and anaphylactoid reactions can involve complement release, but *via* different pathways. In some anaphylactoid reactions, drugs activate the complement cascade either through the classical pathway, by forming a complex with drug-specific, complement-fixing immunoglobulin G or M antibodies, or through the direct interaction with the C_3 component in the alternative pathway. However, more frequently drugs directly induce mediator release from mast cells and basophils.[2] Thus the common distinction for clinicians is between chemically and immunologically mediated reactions. These chemical reactions may accompany parenteral use of drugs such as morphine,[3] atracurium,[4] and vancomycin[5–7] and result in histamine release. The distinction between anaphylac-

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tic and anaphylactoid reactions has important clinical and medicolegal implications. In particular, chemical reactions are potentially preventable (with antihistamines or alteration of infusion rate), but immunologic reactions are less predictable and incompletely prevented by antihistamines. Although tryptase is a marker of immunologic mast cell activation,[8–12] the absence of tryptase release (*in vivo*) during chemically mediated reactions has not been tested.

We used rapid vancomycin administration as a clinically relevant example of chemically mediated histamine release, establishing it as such by direct measurement of histamine levels and the ability of prophylactic intravenous H_1 and H_2 antagonists to attenuate clinical histamine-associated symptoms. The histamine release during rapid vancomycin infusion induces the "red man syndrome," which is characterized by flushing or an erythematous rash over the face, neck, and upper torso; itching; and hypotension, which, in some cases, has manifest as life-threatening shock.[13] Although anaphylactoid reactions have been attenuated by pretreatment with H_1 and H_2 antihistamines in previous studies,[14,15] such pretreatment has not been tested with rapid vancomycin infusion.

Active tryptase most likely is a homotetramer of β-tryptase subunits that is stored in secretory granules and released during degranulation. α-Tryptase is an inactive monomer found in cytosol and continuously secreted by resting mast cells.[16] α-Tryptase is present in normal blood and probably reflects the total body burden of mast cells, because it is markedly elevated in systemic mastocytosis. β-Tryptase is only detected in the blood during systemic anaphylactic reactions and is a marker of mast cell activation.[12–14] Tryptase may be released by degranulation (β-tryptase) or leakage (α-tryptase). The traditional assay for tryptase determination was modified recently. The previous immunoassay (Riact; Pharmacia and Upjohn AB, Uppsala, Sweden) measured only β-tryptase,[17] whereas the new immunoassay (Unicap; Pharmacia and Upjohn AB) measures both α- and β-tryptase.[18] We performed a prospective, randomized, double-blinded, placebo-controlled clinical trial and used the new α-β assay to measure plasma tryptase levels during *in vivo* anaphylactoid reactions induced by rapid vancomycin infusion. Because previous research has shown that tryptase is elevated in immunologic reactions, the demonstration of the lack of tryptase release in this histamine-mediated chemical reaction potentially would allow this assay to distinguish chemical from immunologic reactions.

Methods

Patients

After we received approval from our institutional review board and informed consent, 40 patients who required antibiotic prophylaxis for elective joint replacement surgery received either intravenous antihistamines or placebo before vancomycin infusion. Inclusion criteria were age >18 yr and preoperative American Society of Anesthesiology physical status 1 to 3. Excluded were pregnant or breast-feeding women; patients who had ingested antihistamine-containing products within 7 days before the study; and patients with a history of significant cardiac dysfunction (arrhythmia or ischemic heart disease), atopy, severe asthma, glaucoma, symptomatic benign prostatic hypertrophy, or previous reactions to vancomycin infusion.

Protocol

The patients were allocated by a table of random numbers to receive either antihistamines or placebo on the morning of the operation. One intravenous cannula (16 gauge) was inserted in each arm to administer drugs and sample blood. Coded premedication solutions (the H_1 antagonist diphenhydramine and the H_2 antagonist cimetidine) or equivalent volumes of 0.9% saline prepared by an unblinded observer were infused serially over 3 min, starting 10 min before the infusion of vancomycin. The antihistamines and placebo solutions appeared identical. Vancomycin (1 g in 60 ml of 0.9% saline solution, or 16.7 mg/ml) was infused over 10 min (100 mg/min) *via* a volumetric pump (model AS40A infusion pump; Baxter Healthcare Corp., Deerfield, IL).

The vancomycin infusion was discontinued temporarily if the mean blood pressure (BP) decreased by ≥20% or the patient could not tolerate the itching. Hypotension was treated with boluses of 0.9% saline, phenylephrine, or both at the discretion of a physician blinded to the premedication solutions. Resuscitation equipment was available at the bedside. The unblinded observer revealed the code if the physician requested antihistamines. Patients who had been premedicated with placebo then received rescue medications of intravenous diphenhydramine (50 mg) and cimetidine (300 mg). Once the symptoms resolved or BP stabilized, or both, the vancomycin infusion was begun again at the customary rate.

Measurements

Hemodynamic and clinical variables were measured every minute starting 10 min before and up to 10 min after the vancomycin infusion. Heart rate, supine BP, and cutaneous manifestations were recorded. The heart rate was measured by continuous electrocardiogram. Arterial BPs (systolic, diastolic, and mean) were measured by an oscillometer (Dinamap; Critikon, Johnson & Johnson Medical Instrumentation, Tampa, FL). Patients were questioned by a blinded observer about the presence and intensity of itching 0, 2, 4, 6, 8, and 10 min after the vancomycin infusion was started. Rash manifestations were graded by the same investigator. The severity of rash was classified *a priori* as mild, moderate, or severe from a combined score of the distribution of erythema (local, regional, or systemic) and the degree of redness (0-3^+). Distribution was defined as local (face and neck), regional (upper torso, face, and neck), or systemic (whole body). The final severity ratings were defined as no reaction, mild (local or regional 1^+), moderate (local 2^+ or 3^+), or severe (regional or systemic 1^+-3^+). Changes in mean BP were considered clinically significant if they decreased 20% or more from baseline values.

Blood samples for histamine and tryptase determination of 5-ml volumes were collected in glass vacuum tubes containing EDTA and immediately placed on ice. Samples were taken at baseline and 2, 5, 10, and 20 min after the start of the vancomycin infusion. Plasma was separated by centrifugation at 3,500*g* for 15 min and stored at -20°C until the assay.

Plasma histamine concentrations were determined (duplicate determinations) by the radioimmunoassay method (Immunotech Histamine Radioimmunoassay Kit, Westbrook, ME)[19] with a sensitivity of 0.025 ng/ml. Intra- and interassay variations were 6% and 10%, respectively. Prepared standard curves were linear over the range of 1 to 30 nM, with coefficients of variation of $<10\%$.

Plasma tryptase levels were determined (single determinations) using the UNICAP automated apparatus and the UNICAP tryptase fluoroenzyme immunoassay. The intra- and interassay coefficients of variation were 4.3% and 3%, respectively, with a detection limit of 1 ng/ml.

Ethical Considerations

One aspect of this study that must be addressed is the fact that we were intentionally inducing adverse reactions in patients. Several constraints were imposed by our institutional review board. First, to ensure patient safety, these reactions were induced under the supervision of qualified personnel in a controlled setting. Second, patients were informed specifically about all the risks of hypotension before they gave consent. Third, restrictions were imposed on us such that a study member not involved in the patients' direct clinical care discussed the consent issue with each patient to remove any element of coercion. Finally, an outside monitor was appointed. In granting permission, the institutional review board recognized that reactions to vancomycin can and do occur within clinical practice as the ability to give this drug during a 1-h period, as recommended, often does not exist. No adverse sequelae were noted in any of the patients.

Data Analysis

Demographic characteristics, histamine and tryptase levels, and clinical symptoms were assessed using Fisher's exact test or the Student's *t* test, as appropriate. Changes in tryptase levels were assessed using the paired *t* test. The effect of treatment on the severity of cutaneous reactions was assessed with ordered logistic regression. Univariate and multivariate logistic regression models were used to quantify the association of clinical symptoms with treatment and serum levels (Stata 5.0; Stata Corporation, College Station, TX). All probability values are two sided.

Results

The treatment and placebo groups were similar with respect to patient age, sex, height, and weight (table 1).

Rash developed in all of the patients who received the placebo compared with 63% (12 of 19) of the treated patients (table 1). Itching occurred more frequently in the patients who received the placebo ($P = 0.008$). Four patients who received the antihistamines experienced no rash or itching.

In 2 of 19 patients given antihistamines and 12 of 19 patients given placebo, decreases in mean BP were clinically significant. Phenylephrine was administered to one treated patient and to eight placebo patients. In the two treated patients with clinically significant hypotension, the mean change in BP did not exceed 20%. However, among placebo patients, the maximal decrease in mean BP was $>40\%$ in three patients, 26-30% in six patients, and 20-25% in another three patients. Two patients (one from each group) were excluded from analysis because hemodynamic data were missing.

Table 1. Intravenous Antihistamine Prophylaxis *versus* Placebo for Rapid Vancomycin Infusion

	Antihistamine (n = 19)	Placebo (n = 19)	*P*
Gender (M/F)	10/9	10/9	NS
Age (yr) [median (range)]	53 (29–83)	63 (40–84)	NS
Weight (kg)	85.48	84.74	NS
Height (cm)	168.9	169.7	NS
Effect			
Cutaneous (total)	12	19	0.004
Blood pressure decrease ≥ 20%	2	12	0.002
Vancomycin stopped	2	11	0.005
Levels			
Histamine			
Baseline (mean ± SD, ng/ml)	0.2 ± 0.2†	0.2 ± 0.1‡	0.94
Peak (mean ± SD, ng/ml)	4.7 ± 2.4†	3.5 ± 3.4‡	0.23
Tryptase*			
Baseline (mean ± SD, ng/ml)	5.9 ± 5.4	4.8 ± 2.7	NS
Peak (mean ± SD, ng/ml)	5.9 ± 5.2	5.4 ± 2.8	NS
Post (mean ± SD, ng/ml)	5.2 ± 3.4	5.3 ± 3.3	NS
Δ-tryptase (mean ± SD, ng/ml)	(−) 0.004 ± 0.7	0.5 ± 1.5	NS

Δ-tryptase = the difference between the peak and baseline tryptase levels for each patient.

* Antihistamine group (n = 18), placebo group (n = 17).

† Baseline *versus* peak, $P < 0.0001$.

‡ Baseline *versus* peak, $P = 0.0002$.

Vancomycin infusion was discontinued for 2 of 19 treated patients and for 11 of 19 placebo patients. The infusion was stopped for intolerable itching in one treated and in two placebo patients and for hypotension in one treated and in nine placebo patients. In all of these cases, the vancomycin infusion was begun again at the recommended rate of infusion without any further adverse reactions.

Baseline histamine levels were within normal limits (0.19 ± 0.08 ng/ml) using this method[19] and did not differ between the treatment and placebo groups. Peak histamine levels were approximately 10 times normal and did not differ between treatment and placebo groups (table 1).

Three patients had baseline tryptase levels <1 ng/ml, the lowest calibrator value, and were excluded from analysis. Baseline tryptase levels did not differ between the treatment and placebo groups (table 1). The mean baseline, peak, and postvancomycin tryptase levels were comparable for the treatment group, the placebo group, and for all patients (table 1). The differences between peak and baseline tryptase levels for each patient, designated Δ-tryptase, were not statistically significant (table 1). This study had significant power to detect changes in tryptase levels. The 99% confidence intervals for the antihistamine and placebo groups, respectively, were −0.5 to +0.5 and −0.6 to +1.6.

To determine the relation between Δ-tryptase levels and the presence of abnormal peak histamine levels, pretreatment with intravenous antihistamines, or both, we performed univariate and multivariate logistic regression analysis. Abnormal peak histamine levels were defined as plasma levels ≥1 ng/ml. Outcome variables included hypotension (decreased mean arterial BP ≥20%), moderate-to-severe rash, and a decision to stop the infusion. Univariate effects of abnormal peak histamine levels were evaluated in the placebo group only; Δ-tryptase and antihistamine pretreatment were evaluated in all patients.

The results of the univariate analysis (table 2) indicate that abnormal histamine levels showed a trend toward increased risk of the adverse events of the red man syndrome. Δ-tryptase was independent of increased histamine levels, antihistamine pretreatment, or both, as well as the common manifestations of vancomycin-induced anaphylactoid reactions. Multivariate analysis (not shown) yielded similar findings.

Discussion

Anaphylaxis may be seen in as many as 1 in 3,000 hospital admissions[20] and may account for >500 deaths each year.[21] Aside from patient history, there are no known characteristics that identify persons at risk for anaphylaxis. The dramatic increase in latex-induced anaphylaxis was highlighted in a recent French study in which the incidence of perioperative reactions increased from 0.5% in 1990 to 19% by 1994.[22] Other perioperative reactions, known as anaphylactoid reactions, involve drugs such as morphine, meperidine, and vancomycin, which induce chemically mediated histamine release that depends on the speed and the dose of administration. Severe chemically mediated reactions may be clinically indistinguishable from anaphylaxis but are more common.[15] Some agents, such as muscle relaxants, are associated with both syndromes. The ability to distinguish anaphylactic from anaphylactoid reactions would aid in investigations of shock or sudden unexpected deaths, appropriately classify patients as allergic to an agent, and clarify epidemiologic or medicolegal issues.

During anaphylactic reactions, activated mast cells co-

Table 2. Logistic Regression Analysis of Histamine, Δ-Tryptase, and Clinical Manifestations of Red Man Syndrome during Rapid Vancomycin Infusion

Independent Variables	Dependent Variables								
	Hypotension			Rash			Stopped Infusion		
	Odds Ratio	CI	*P* Value	Odds Ratio	CI	*P* Value	Odds Ratio	CI	*P* Value
Abnormal histamine	8.25	0.65, 104.19	0.10	19.50	1.30, 292.75	0.03	6	0.49, 73.45	0.16
Δ-Tryptase	1.44	0.78, 2.67	0.24	1.47	0.75, 2.86	0.26	1.31	0.74, 2.32	0.36
Antihistamine pretreatment	0.07	0.01, 0.39	0.002	0.21	0.05, 0.83	0.03	0.09	0.02, 0.48	0.005

Δ-tryptase = the difference between the peak and baseline tryptase levels for each patient; CI = confidence interval.

release histamine and tryptase. Tryptase levels after bee sting–induced anaphylaxis correlated with clinical severity and hypotension.[23] Anaphylactic reactions to neuromuscular blockers, confirmed by skin tests and specific immunoglobulin E antibodies, are associated with increased concentrations of plasma histamine and tryptase.[10–12,24] A recent study of 350 patients experiencing life-threatening reactions under anesthesia emphasized the value of an increased tryptase level as a consistent and reliable indicator of anaphylactic reactions.[9] But no clinical data exist to show that chemically mediated reactions are not associated with an increased tryptase level. Such confirmation is vitally important from patient care and medicolegal perspectives. Using this new assay for α- and β-tryptase, we determined that tryptase release does not occur in vancomycin-induced anaphylactoid reactions.

Our data confirm that the red man syndrome is a manifestation of chemically mediated histamine release, as it is largely attenuated by prophylactic intravenous H_1 and H_2 antihistamines. Univariate and multivariate logistic regression analysis of our data (table 2) showed that elevated plasma histamine levels (>1 ng/ml) during rapid vancomycin infusion conveyed an eightfold increase in the likelihood of hypotension, a 20-fold likelihood of rash, and a nearly sixfold likelihood of stopping the rapid infusion. Antihistamine protection of histamine-mediated symptoms was virtually complete for hypotension (14-fold) and stopped infusion (12-fold) and significant but less adequate for rash.

Despite 10-fold or greater elevations in plasma histamine and associated clinical symptoms, tryptase levels remained within normal limits in all patients. Both univariate (table 2) and multivariate (not shown) analyses confirmed that the Δ-tryptase failed to predict hypotension, cutaneous manifestations, or the decision to stop the infusion. We saw no increases in tryptase levels either at the times of peak histamine elevations or in later samples harvested 10 min after the rapid vancomycin infusion was complete. The lack of significant tryptase elevations in any of the 40 patients receiving rapid vancomycin infusions established the exceptionally robust confidence intervals.

The use of tryptase as a marker for anaphylaxis has two primary advantages over plasma histamine: time course and stability. Based on evaluations with the β-tryptase assay, levels increase by 15 min after antigen presentation and decline in apparent first-order kinetics with a plasma half-life of approximately 2 h.[25] Blood for tryptase determination need not be harvested during periods of acute hemodynamic instability to identify an immune reaction. Tryptase is present in negligible amounts (0.04 pg/cell) in basophils and has not been detected in non—mast cells in the lung, skin, bowel, or peripheral blood.[9,26] Tryptase remains stable in plasma or sera stored at −20°C for long periods (>10 yr in one study)[27] and is relatively stable in postmortem blood.[28] Freezing and thawing have a negligible effect on tryptase determination.

The previously available commercial assay for tryptase measured β-tryptase,[17] whereas the new assay measures α- and β-tryptase.[18] α-Tryptase appears to be the primary form of human tryptase present in the blood at baseline in both healthy persons and in those with systemic mastocytosis.[18] β-Tryptase is the predominant form in preparations from mast cells in the skin, lung, and blood during systemic anaphylaxis. The α-β assay may be more sensitive for severe anaphylactic reactions,[17] because a greater portion of the total tryptase present in solution is detected but may be of less value for mild reactions because of the overlap of baseline α-tryptase levels with modest elevations in β-tryptase (D Laroche, J Sainte-Laudy, unpublished study).

The observation that tryptase levels in these reactions

did not increase, were independent of histamine release, and did not predict symptoms shows that plasma tryptase levels are not increased in chemically mediated reactions. This observation, when taken in concert with evidence of release in immune reactions, suggests that the new tryptase assay may be able to distinguish between anaphylactic and anaphylactoid reactions.

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