

Coagulation cascade and fibrinolysis in patients with multiple-drug allergy syndrome

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Background: Previous studies have shown that patients with multiple-drug allergy syndrome (MDAS) frequently have positive autologous serum skin test results, similar to patients with chronic urticaria (CU). Recent investigations have found that patients with CU show signs of thrombin generation and activation of the tissue factor pathway of the coagulation cascade.

Objective: To study thrombin generation and fibrinolysis in patients with MDAS.

Methods: Nine patients with MDAS underwent autologous plasma skin testing (APST) and measurement of plasma prothrombin fragment F_{1+2} and D-dimer levels. Furthermore, the basophil histamine-releasing activity of plasma from patients with MDAS was evaluated. Plasma samples from 74 healthy control subjects and 13 patients with CU were used as negative and positive controls, respectively.

Results: All 9 patients with MDAS had positive APST results, and 7 showed elevated plasma levels of fragment F_{1+2} . In patients with MDAS, the median F_{1+2} level (339 pmol/L; interquartile range [IQR], 250–401 pmol/L) significantly exceeded that in healthy controls (159 pmol/L; IQR, 123–196 pmol/L) ($P = .001$) but did not significantly differ from that in controls with CU (292 pmol/L; IQR, 182–564 pmol/L; $P = .38$). Plasma D-dimer levels were normal in all the patients with MDAS and were significantly lower than in controls with CU ($P = .009$). Finally, the histamine-releasing activity of plasma from patients with MDAS was significantly increased and correlated with F_{1+2} levels ($r = 0.68$; $P = .04$).

Conclusion: Positive APST results and thrombin generation indicate a common physiopathologic background in MDAS and CU. The lower D-dimer levels suggest that fibrinolysis occurs less intensely in MDAS than in CU.

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INTRODUCTION

The so-called multiple-drug allergy syndrome (MDAS) is a clinical condition characterized by a propensity to react against different, chemically unrelated drugs. The most frequent causes of MDAS are antibacterial drugs^{1–3} and nonsteroidal anti-inflammatory drugs,^{4,5} but other drug classes may be involved in this condition as well. In our experience, in most instances the syndrome presents clinically as acute urticaria with or without angioedema after administration of the offending compounds, but different immediate-type or delayed-type rashes, including Stevens-Johnson syndrome, anaphylaxis, serum sickness-like reactions, and immune cytopenias, have also been reported. Because, except for some cases of positive skin and serologic reactivity to penicillin reagents, in patients with MDAS results of skin tests with the offending compounds and specific antibody determinations are negative, and reactions may occur after the first admin-

istration of a certain drug class, most of these adverse drug reactions must be regarded as pseudoallergic. Patients with MDAS are frequently otherwise healthy and do not have a history of spontaneous rashes or an increased prevalence of a personal or familial history of atopic diseases and do not show any identifiable underlying disease. In most cases MDAS is diagnosed on the basis of clinical history or the direct observation of adverse reactions to 1 or more drugs other than those reported as offending by history during oral tolerance tests.

The pathogenic mechanisms underlying MDAS are still unclear; however, recent studies have found that patients with this disorder frequently show a wheal-and-flare reaction on intradermal injection of autologous serum (autologous serum skin test [ASST]).⁶ The ASST has been used for approximately 2 decades as a means to detect autoreactivity (ie, the presence of circulating histamine-releasing factors) in patients with chronic urticaria (CU).⁷ The presence of circulating autoantibodies to the high-affinity IgE receptor (FcεRI) or to IgE has been firmly demonstrated⁸ in patients with CU, and positive ASST results have been ascribed to their presence in approximately one-third of patients.⁹ In the remaining cases the exact nature of histamine-releasing factors involved in skin reactivity to autologous serum is still debated, and IgG-depleted sera from patients with CU maintain the ability to induce a wheal-and-flare reaction on intradermal injection.¹⁰ Recently, we found that in patients with CU the intradermal injection of autologous plasma anticoagulated with

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sodium citrate (autologous plasma skin test [APST]) scores positive in approximately 90% of patients (vs approximately 50% of ASST).¹¹ This observation suggested a possible role of factors much more abundantly present in plasma than in serum in the wheal-and-flare reactions induced by autologous plasma. In effect, when we studied the clotting cascade in these patients we demonstrated that thrombin generation occurs in patients with CU, as shown by significantly elevated plasma levels of prothrombin fragment F₁₊₂. Such fragment is a polypeptide of approximately 34 kDa that is released into the circulation during the activation of prothrombin to thrombin by factor Xa.¹² In subsequent experiments we found that thrombin generation results from activation of the tissue factor pathway of the coagulation cascade.¹³ The putative role of thrombin is suggested by experimental animal models, where thrombin induces edema through an increase in vascular permeability,^{14,15} which can be obtained by a direct effect of thrombin on endothelial cells¹⁶ and indirectly by a thrombin-related release of inflammatory mediators, an effect that is reduced by antihistamines and totally abolished if mast cell granules are depleted.¹⁵ Furthermore, thrombin triggers mast cell degranulation¹⁷ and may activate protease-activated receptor 1 on mast cells.¹⁸ Finally, thrombin can generate C5a in the absence of C3, thus bypassing the whole first part of the complement cascade.¹⁹ Nonetheless, direct evidence that thrombin is a histamine-releasing factor in humans is still lacking. Based on these observations, we looked for signs of thrombin generation and studied the coagulation cascade in patients with MDAS.

PATIENTS AND METHODS

Patients

This study was conducted on 9 consecutive women with MDAS (mean age, 50.1 years; age range, 36–76 years). The MDAS was diagnosed in the presence of an unequivocal history of multiple acute urticaria episodes after exposure to chemically unrelated drugs or, alternatively, if acute urticaria developed during oral tolerance tests with alternative substances in individuals with a history of single-drug-induced urticaria. The clinical features of the study patients are sum-

marized in Table 1. Six patients had a clinical history of multiple-drug intolerance, and 3 had a history of single-drug intolerance, with positive oral tolerance test results for alternative substances. Offending drugs induced acute urticaria in all cases. Reported offending drugs included amoxicillin (n = 8), macrolides (n = 3), quinolones (n = 2), nonsteroidal anti-inflammatory drugs (n = 2), sulfamethoxazole-trimethoprim, doxycycline, gentamicin, and metoclopramide (n = 1 each). No patient had a history of CU or of spontaneous whealing. Three patients were atopic and had respiratory allergy, as shown by clinical history and positive skin prick test reactions to a large panel of seasonal and perennial airborne allergens. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the local ethical committee. All the participants gave written informed consent before enrollment.

Oral Tolerance Tests

Based on clinical history, all the patients underwent single-blind, placebo-controlled oral tolerance tests with alternative substances at San Carlo Clinic, as previously described.⁴ In all cases, only the appearance of frank urticaria with or without angioedema was considered to be a positive reaction. All the patients (except 1 with a history of multiple-drug intolerance) did not tolerate at least 1 of the alternative antibiotics tested on oral challenge tests, and 1 patient did not tolerate aspirin either (Table 1). At the end of the oral challenge sessions all the patients fulfilled the diagnostic criteria for MDAS; at least 1 safe alternative antibiotic was detected in 7 patients, whereas 2 tolerated alternative drugs only while taking an antihistamine (cetirizine, 10 mg/d, in both cases).

Skin Tests

The APST was performed in all 9 patients with MDAS; 5 of these patients also underwent ASST. Intradermal tests were performed as previously described.¹¹ A skin prick test with histamine, 10 mg/mL, and an intradermal test with 0.05 mL of saline were used as positive and negative controls, respectively. Ten individuals with allergic rhinitis and 5 with contact dermatitis underwent APST as controls.

Table 1. Clinical Features of 9 Women With Multiple-Drug Allergy Syndrome

Patient No.	Patient age, y	APST results	ASST results	History	Intolerance (challenge)	Tolerance (challenge)
1	42	4+	4+	A	C	D, Cip
2	57	3+	Negative	A, D, NSAIDs	Cip, C, S	Only with a-H ₁
3	46	4+	Negative	S, N	Cip	A, C
4	63	3+	Negative	C	A, S, D, ASA	Cip
5	76	3+	3+	A	C, Cip	Lin, D
6	41	4+	ND	A, C, M	None	Cip, D
7	49	4+	ND	A, Cip	C, D	S
8	41	3+	ND	A, G, E	D	Cip
9	36	4+	ND	A, Lo	C, S	D, C (with a-H ₁)

Abbreviations: A, amoxicillin; a-H₁, antihistamine; APST, autologous plasma skin test; ASA, aspirin; ASST, autologous serum skin test; D, doxycycline; C, clarithromycin; Cip, ciprofloxacin; E, erythromycin; Lin, lincomycin; Lo, lomefloxacin; M, metoclopramide; N, naproxen; ND, not determined; NSAIDs, several nonsteroidal anti-inflammatory drugs; S, sulfamethoxazole-trimethoprim.

Fragment F_{1+2} and D-Dimer Measurement

Fragment F_{1+2} was measured in sodium citrate–anticoagulated plasma samples by means of a sandwich enzyme-linked immunosorbent assay (Enzygnost F_{1+2} ; Behring Diagnostics GmbH, Frankfurt, Germany). Intra-assay and interassay coefficients of variation were 5% and 8%, respectively. D-dimer was measured in sodium citrate–anticoagulated plasma samples by means of enzyme-linked immunosorbent assay (Zymutest D-dimer; Hyphen BioMed, Neuville sur Oise, France) according to the manufacturer's instructions. The intra-assay and interassay coefficients of variation were 10% and 15%, respectively. Plasma levels less than 229 pmol/L and less than 4.00 nmol/L were considered normal for fragment F_{1+2} and D-dimer, respectively. Plasma samples from 74 healthy controls and from 13 patients with CU were used as negative and positive controls, respectively.

Basophil Histamine-Release Assay

Leukocyte suspensions from healthy blood donors were prepared by means of dextran sedimentation of peripheral venous blood, anticoagulated with 0.01M EDTA, and mixed with 6% dextran in saline solution (Plander 70; Fresenius Kabi Italia, Isola della Scala, Verona, Italy) and 30-mmol/L dextrose (Sigma-Aldrich Corp, St Louis, Missouri). The cells were allowed to settle for 60 to 90 minutes at room temperature. Then, leukocyte-rich plasma was aspirated and centrifuged at 300g for 15 minutes at 4°C, and the cell button was washed twice in Tyrode buffer, pH 7.4, containing 140mM sodium chloride, 5.5mM dextrose, 2.7mM potassium chloride, 0.36mM sodium dihydrogen phosphate, and 12mM sodium bicarbonate. Leukocytes (with approximately 7×10^4 basophils) were then resuspended in a 100- μ L volume of Tyrode buffer with 1.8-mmol/L calcium chloride and 0.5-mmol/L magnesium chloride and incubated with 100 μ L of the serum under examination, making a final volume of 200 μ L. After incubation for 40 minutes at 37°C, the reaction was stopped by the addition of 800 μ L of ice-cold buffer solution and centrifugation at 1,000g for 10 minutes at 4°C. After centrifugation, the supernatants were aspirated, mixed with an equal volume of perchloric acid, 6%, and centrifuged at 1,000g for 10 minutes at 4°C. Histamine concentration in the supernatants was measured using an automated fluorometric method, according to the method of Ruff et al.²⁰ Spontaneous histamine release was evaluated by measuring histamine concentration in the supernatant of unstimulated cells incubated for 40 minutes at 37°C. Total histamine content was obtained by adding 100 μ L of perchloric acid, 6%, to 100 μ L of cell suspension. Net histamine release was calculated as the percentage of total histamine content after subtraction of spontaneous release. A 5% release cutoff value was used. Plasma samples were tested using leukocyte suspensions from 3 healthy donors whose basophils were previously shown to release 30% of total histamine content on challenge with an optimal dose of goat polyclonal anti-human IgE (Sigma-Aldrich Corp), 10 μ g/mL. One assay per plasma sample was performed to detect histamine-releasing activity. Histamine-

release assay was performed using the same plasma samples used for APST.

RESULTS

Skin Tests

Results of APST were positive in all 9 patients with MDAS, and results of ASST were positive in 2 of 5 patients with MDAS (Table 1); saline did not induce any skin reaction in any patients with MDAS. No skin reactivity on APST was observed in the 15 controls.

Fragment F_{1+2} and D-Dimer Measurement

Fragment F_{1+2} plasma levels in patients and controls are shown in Figure 1. In patients with MDAS, the median F_{1+2} level (339 pmol/L; interquartile range [IQR], 250–401 pmol/L) was significantly higher than in healthy controls (159 pmol/L; IQR, 123–196 pmol/L) ($P = .001$) but not significantly different from controls with CU (292 pmol/L; IQR, 182–564 pmol/L; $P = .38$). Fragment F_{1+2} plasma levels were clearly elevated in 7 of 9 patients (78%) (Fig 1).

D-dimer plasma levels in patients and controls are shown in Figure 2. In patients with MDAS, the median D-dimer plasma level (1.19 nmol/L; IQR, 1.01–1.44 nmol/L) did not differ from that in healthy controls (1.42 nmol/L; IQR, 0.96–1.63 nmol/L) but was significantly lower than in controls

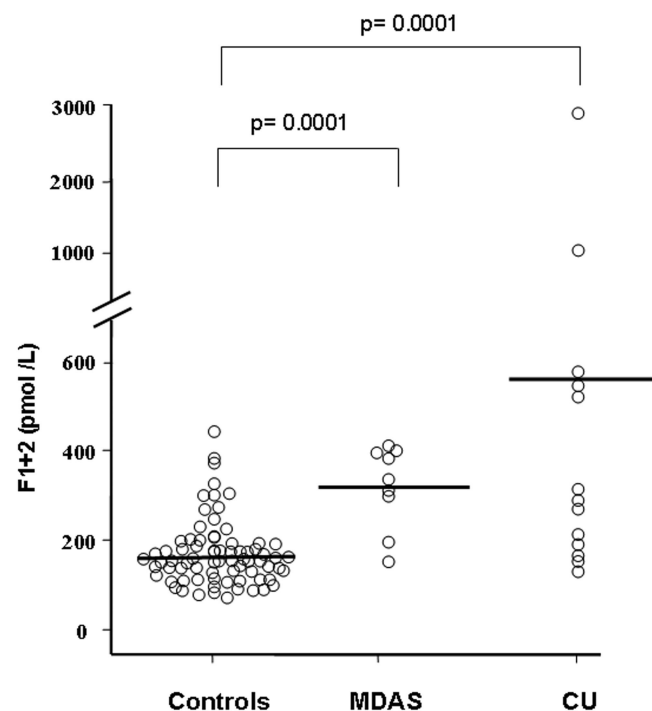


Figure 1. Plasma levels of prothrombin fragment F_{1+2} in healthy control subjects, patients with multiple-drug allergy syndrome (MDAS), and controls with chronic urticaria (CU). Horizontal lines represent median values; white circles, individual participant values. The F_{1+2} plasma levels were higher in patients with MDAS and in those with CU than in healthy controls.

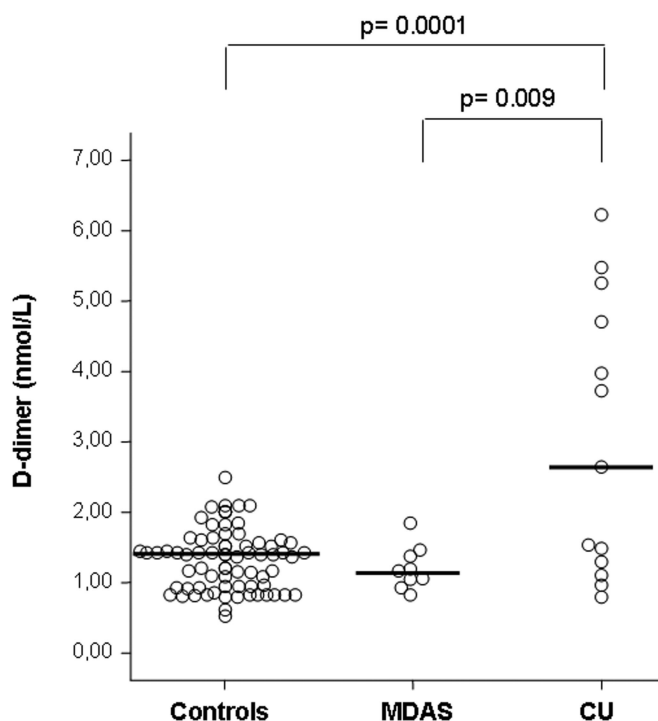


Figure 2. Plasma levels of D-dimer in healthy control subjects, patients with multiple-drug allergy syndrome (MDAS), and controls with chronic urticaria (CU). Horizontal lines represent median values; white circles, individual participant values. D-dimer plasma levels were higher in patients with CU, but not in patients with MDAS, than in healthy controls.

with CU (2.67 nmol/L; IQR, 1.22–5.00 nmol/L) ($P = .009$). No patient with MDAS showed elevated D-dimer levels (Fig 2).

Basophil Histamine-Release Assay

Plasma samples from 5 of 9 patients with MDAS (56%), 4 of 13 controls with CU (31%), and 0 of 20 healthy controls induced significant histamine release ($P = .001$ for patients with MDAS vs healthy controls) (Fig 3). In patients with MDAS, plasma histamine-releasing activity was significantly correlated with plasma F_{1+2} levels ($r = 0.68$; $P = .04$) but not with D-dimer levels.

DISCUSSION

One previous study⁶ already showed that MDAS and CU share a high prevalence of positive ASST results. Thus, based on our recent experience in patients with CU^{12,13} demonstrating that patients with this condition show signs of thrombin generation, we hypothesized that patients with MDAS might show the same disorders and performed APST and plasma F_{1+2} prothrombin fragment and D-dimer measurements in patients diagnosed as having MDAS on the basis of clinical history and oral tolerance tests with alternative drugs. The findings fully confirmed the preliminary hypothesis in that, similar to patients with CU, all the patients with MDAS had

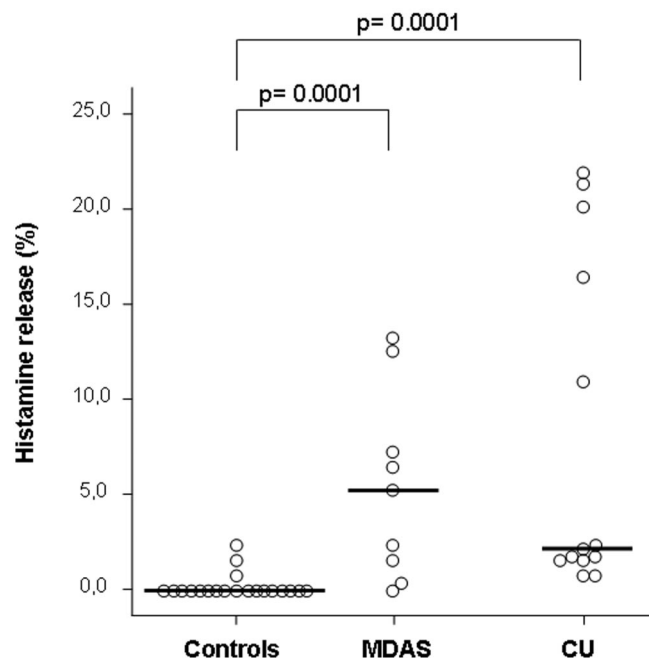


Figure 3. Histamine-releasing activity in serum samples from healthy control subjects, patients with multiple-drug allergy syndrome (MDAS), and controls with chronic urticaria (CU). Results are expressed as net percentage of histamine release from basophils of a healthy donor. Horizontal lines represent median values; white circles, individual participant values. Serum histamine-releasing activity was significantly higher in patients with MDAS and in controls with CU than in healthy controls.

positive APST results and showed signs of thrombin generation in most cases, as demonstrated by the significant increase in fragment F_{1+2} plasma levels. As a difference from patients with CU,¹³ particularly those with severe disease,²¹ D-dimer levels were normal in all 9 patients with MDAS.

Data on human cutaneous mast cells are still missing. However, in animal models, thrombin, an enzyme, increases the permeability of blood vessels,^{14–16} induces mast cell degranulation,¹⁷ and may activate protease-activated receptor 1 on mast cells.¹⁸ The effects of thrombin on vascular permeability are reduced by antihistamines and are totally abolished if mast cell granules are depleted.¹⁵ Recent studies¹⁹ also showed that thrombin can generate C5a in the absence of C3, thus bypassing the entire first part of the complement cascade. In patients with CU, C5a has been shown to amplify IgG-dependent histamine release from basophils.²² Serum histamine-releasing activity was significantly higher in patients with MDAS than in healthy controls, suggesting that these patients have circulating histamine-releasing factors similar to patients with CU. In addition, the positive correlation between F_{1+2} plasma levels and serum histamine-releasing activity indicates that activation of the coagulation cascade and serum capacity to induce histamine release are parallel and probably interrelated phenomena in patients with MDAS.

Based on results from a small group, patients with MDAS seem to have a condition similar to a "latent" form of CU that is not sufficiently active to cause spontaneous wheals or angioedema but is nonspecifically triggered by exposure to chemically unrelated drugs, possibly via a direct histamine-releasing effect of drugs on mast cells and basophils. Mast cells and basophils of patients with MDAS might be in a "preactivated" (ie, particularly prone to degranulate) state at the time when the offending drug is taken; drugs would act as a final trigger for degranulation followed by acute urticaria. The fact that all the patients with MDAS showed normal D-dimer levels suggests that in this condition, activation of the coagulation cascade and fibrinolysis occur at a lower level than in patients with spontaneous CU. In effect, in previous studies^{11,13} we demonstrated that the severity of spontaneous urticaria is associated with the degree of activation of the extrinsic pathway of coagulation. In conclusion, the currently available data suggest that patients with MDAS and those with CU show activation of the coagulation cascade, although the former at a lower level and without fibrinolysis. Further studies on larger groups of patients with MDAS are warranted to confirm the present findings.

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