

Autoreactivity is highly prevalent in patients with multiple intolerances to NSAIDs

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Background: A subset of drug-allergic patients show a marked propensity to react against several, chemically unrelated nonsteroidal anti-inflammatory drugs (NSAIDs). The pathogenesis of such multiple drug reactions is unclear. Approximately 30% of patients with chronic idiopathic urticaria, a condition frequently characterized by autoreactivity on autologous serum skin test (ASST), experience flares of hives after taking chemically unrelated NSAIDs.

Objective: To detect whether a clinically unapparent autoreactivity may represent the nonspecific mechanism facilitating drug-induced histamine release in patients with a history of urticaria/angioedema induced by several, chemically unrelated NSAIDs.

Methods: Thirty-six adults with a history of acute NSAID-induced urticaria (22 with multiple NSAID sensitivity [MNS]; 14 with single NSAID sensitivity [SNS]; and 20 atopic controls without a history of drug allergy) underwent ASST. Sera from 14 MNS and 4 SNS subjects (all ASST-positive) underwent histamine release assay with basophils from normal donors. Sera from five MNS patients were tested on autologous basophils as well.

Results: Twenty of 22 (91%) MNS subjects versus 5 of 14 (36%) SNS subjects were positive on ASST ($P < 0.01$). No atopic control was ASST-positive. Sera from 4 of 14 (29%) MNS patients versus 0/4 SNS subjects ($P = \text{NS}$) induced significant histamine release from basophils of normal donors. The use of autologous basophils did not significantly change these results.

Conclusion: Most patients with multiple NSAID intolerance and approximately one-third of those with single NSAID hypersensitivity are characterized by the presence of circulating histamine-releasing factors. Their nature is still unclear, but the fact that only a minority of sera from ASST+ subjects were able to induce histamine release from normal basophils in vitro suggests that these factors might not differ from those involved in most patients with chronic urticaria. These factors might play a relevant pathogenic role in NSAID-induced urticaria reactions.

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INTRODUCTION

The term “pseudoallergic” identifies drug-induced adverse reactions characterized by clinical manifestations that are suggestive of or compatible with immunopathologic mechanisms but that are known to lack an immune basis.¹ Such reactions probably represent the majority of drug-induced skin disorders seen by allergists. Their patho-

genesis is still obscure; a number of studies tried to identify possible immune-mediated mechanisms, such as immunoglobulin (Ig)E specific for metabolic breakdown products of the parent offending drugs, or specifically sensitized lymphocytes, with little success. A subset of patients with a history of nonsteroidal anti-inflammatory drug (NSAID)-induced urticaria and/or angioedema seem to show a marked propensity to react to several, chemically unrelated compounds.^{2,3} These patients are otherwise normal subjects without a clinical history of chronic or recurrent urticaria, a condition that is frequently associated with multiple

NSAID intolerance,⁴ and have been classified as a specific category in recently proposed classification system.⁵

Mechanisms underlying multiple drug reactivity remain elusive, and the marked differences between offending drugs seem to rule out a possible pathogenesis based upon immunologic recognition of specific chemical structures (ie, cross-reactivity) but rather suggest the existence of nonspecific, patient-related factors leading to direct histamine release from mast cells and/or basophils. Recent studies reported good results both on the primary skin disorder and on NSAID reactivity in some patients with chronic urticaria by leukotriene antagonists,^{6–10} suggesting that inhibition of cyclooxygenase (COX) pathway may play a role in these drug-induced reactions. The recent observation that selective COX-2 inhibitors are well tolerated by a majority of these patients supports this hypothesis.^{11,12} However, some patients experience urticaria even after taking weak COX-inhibitors such as acetaminophen (paracetamol), floctafenine, rofecoxib, and/or nimesulide,^{3,12} suggesting the possible involvement of other pathogenic mechanisms as well. One of these might be a condition characterized by a facilitated histamine release from both mast cells and basophils.

Several studies demonstrated that a high proportion (up to 60%) of patients with chronic idiopathic urticaria show a wheal-and-flare reaction upon intradermal injection of autologous serum.^{13–15} In these patients, serum histamine-releasing activity has long been ascribed to the presence of circulating IgG autoantibodies specific either for the high-affinity IgE receptor, FcεRI, or for IgE; such autoantibodies would be responsible for histamine release from both basophils from normal do-

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nors and human mast cells in vitro.¹⁵⁻²⁰ However, recently, the clinical significance of such autoantibodies has been questioned after it was found that sera from urticaria patients maintain their ability to induce wheal-and-flare reactions after IgG depletion.²¹ Chronic idiopathic urticaria is often associated with multiple NSAID intolerance, and these observations prompted us to investigate whether a clinically unapparent autoreactivity may represent a possible nonspecific mechanism facilitating drug-induced histamine release in patients with a history of urticaria/angioedema induced by several, chemically unrelated drugs but without a history of chronic urticaria.

PATIENTS AND METHODS

Patients

Thirty-six consecutive adult patients (male:female 10:26, age 17 to 68 years, mean age 43.4 years) with an unequivocal history of acute episodes of urticaria, with or without angioedema, after taking NSAIDs were

studied. All these subjects were otherwise healthy, and no one reported spontaneous episodes of urticaria. Twenty-two patients (male:female 4:18, mean age 47.9 years) had a history of multiple NSAID intolerance (multiple NSAID sensitivity [MNS]); 11 of them had a history of urticaria after taking also drugs other than NSAIDs (Table 1). Fourteen consecutive subjects (male:female 6:8, mean age 36.4 years) with a history of single NSAID intolerance (aspirin in 8 cases, pyrazolones and nimesulide in 2 cases each, naproxen and diclofenac in 1 case) were used as positive controls (single NSAID sensitivity [SNS]; Table 2).

Both MNS and SNS subjects presented at this allergy center asking for safe alternative drugs. Therefore, based on clinical history, patients underwent single-blind, placebo-controlled tolerance tests with the aim to detect at least two tolerated compounds. All patients gave an informed written consent before the start of the procedure. Doses and methods used in

oral tolerance tests with alternative drugs have been described elsewhere^{2,3,12}; briefly, doses equivalent to 25, 50, and 100% dose of the challenged drug (aspirin 500 mg, acetaminophen 500 mg, nimesulide 100 mg, floctafenine 200 mg, rofecoxib 25 mg, or tramadol 50 mg) were given 1 hour apart. Placebo or active doses were challenged 1 week apart. Patients were kept under control, for at least 1 hour after the last provocative dose. Results of oral tolerance tests are shown in Tables 1 and 2.

Skin Tests

Both MNS and SNS patients underwent intradermal test with 0.05 mL of sterile, fresh autologous serum (autologous serum skin test [ASST]) and with saline as negative control. A skin prick test (SPT) with histamine 10 mg/mL, performed using a 1-mm tip, sterile, disposable lancet was used as positive control. Readings were taken at 15 minutes. The wheal-and flare reaction induced by autologous serum was

Table 1. Offending Compounds in Patients with Multiple Drug Allergy

No.	ASA	Propionic A.D.	Aryl-acetates	Piroxicam	Pyrazolones	Morniflumate	Paracetamol	Nimesulide	Floctafenine	Rofecoxib	Tramadol	Other offending drugs
1	U							Uc		Tc		Metisoprinole, antibiotics (P, Q, T)
2		U	U				U	U		Uc		Antibiotics (M, Q, C, T, R)
3	U			U	U		U	U	Tc	Tc	T	Nomegestrol; acetylcysteine, antibiotics (P, M, T)
4					T		U	U				β -lactams
5	U	U					Tc	Tc	Tc	Tc	Uc	Antibiotics (P, M, T, L)
6	U				U			U				β -lactams
7	U	U			U		Tc	U	Tc			Antibiotics (Q, C)
8	U				U			Tc		Tc	Tc	Antibiotics (M, C)
9	U		U		U		T	U		Uc	Uc	Antibiotics (P, M)
10	Uc		U				Tc	Tc		Tc	Tc	Antibiotics (P, Q)
11	U						U			Tc		
12	U			U	U		Tc	Uc				
13	U	U			U		U	Uc		Tc		
14	U						U	T	Tc			Blueberry extract
15	U				U		T	U		Tc	Tc	
16	U				U		Tc			Tc		
17	U	U		U			Tc	Tc				
18	U	U						T				
19				U		U	Tc	U		Tc		
20			U		U		Tc	Tc		Tc	Tc	
21		U		U			Tc	U		Tc	Tc	
22	U		U	U			Tc	U		Tc	Tc	

U: Urticaria; T: Tolerated; c: Drug challenged. Arylacetates include: diclofenac, tolmetine, and ketorolac. M: Macrolides; Q: Quinolones; T: Tetracyclines; R: Rifampicin; L: Lincomycin; P: Penicillins; C: Cotrimoxazole.

Table 2. Offending Drugs in Patients with Single Drug Allergy

No.	ASA	Propionic A.D.	Diclofenac	Paracetamol	Nimesulide	Pyrazolones	Rofecoxib	Other offending drugs
1	Tc			U	T			Amoxicillin
2	Tc			Tc		U		Macrolides
3	U			Tc	Tc			
4	U				Tc			
5	U			Tc				
6	Tc			Tc	U			
7	T	U						
8	U		T	Tc	Tc	T		
9	U			Tc			Tc	
10	U					T		
11	U						Tc	
12	Tc		U					
13	Tc					U		
14	U			Tc	Tc		Tc	

graded as 2+ (50 to 100%), 3+ (100%), or 4+ (>100%) by comparison with the histamine wheal, as previously described.²² To control the reported inhibitory effect of heparin on histamine release *in vivo*,²³ in four patients intradermal test with 0.05 mL of autologous heparinized plasma was carried out as well. To evaluate the specificity of ASST, 20 atopic controls without a history of chronic urticaria or of drug allergy underwent ASST.

Basophil Histamine Release Assay

Sera from 14 MNS patients and from 4 SNS subjects (all ASST+) were tested for their capacity to induce histamine release from basophils of normal donors. Further, the histamine-releasing activity of sera from five patients was evaluated on autologous basophils as well.

Leukocyte suspensions from both normal donors and patients were prepared by dextran sedimentation of peripheral venous blood, anticoagulated with 0.01 M EDTA, and mixed with 6% dextran in saline solution (Solplex 70, Sifra, Verona, Italy) and 30 mmol/L dextrose (Sigma Chemicals, St Louis, MO). The cells were allowed to settle for 60 to 90 minutes at room temperature, according to Lichtenstein and Osler.²⁴ The leukocyte-rich plasma was aspirated and centrifuged at 300 *g* for 15 minutes at 4° C, and the cell button was washed twice in Tyrode buffer, pH 7.4, containing (mM) 140

NaCl, 5.5 dextrose, 2.7 KCl, 0.36 NaH₂PO₄, and 12 NaHCO₃. Leukocytes (with approximately 7×10^4 basophils) were then resuspended in 100 μ L volumes of Tyrode buffer with 1.8 mmol/L CaCl₂ and 0.5 mmol/L MgCl₂, and incubated with 100 μ L of the serum samples, making a final volume of 200 μ L. After incubation for 40 minutes at 37° C, the reaction was stopped by addition of 800 μ L of ice-cold buffer solution and centrifugation at 1,000 *g* for 10 minutes at 4° C. The supernatants were aspirated and frozen at -20° C until assayed. Histamine concentration in the supernatants was measured by radioimmunoassay, using a commercially available kit (Immuno-tech, Marseille, France). 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 heating the cells at 85° C for 1 hour. Net histamine release was calculated as percentage of total histamine content, after subtraction of spontaneous release. A 5% release cut-off value was used. Sera were tested with basophils from three normal donors. As control, basophils were stimulated with an optimal dose (10 μ g/mL) of goat polyclonal anti-human IgE (Sigma Chemicals). Anti-IgE induced 48.2, 60.6, and 20% histamine release from basophils of the three donors, respectively.

Statistical Methods

Proportions were compared by χ^2 test with Yates' correction. Probability (*P*) values less than 0.05 were considered significant.

RESULTS

Skin Tests

Twenty of 22 (91%) MNS patients versus 5 of 14 (36%) SNS subjects were positive on ASST (*P* < 0.01; Table 3). Of the remaining 2 MNS patients, 1 was negative, and 1 was not valuable because of dermatographism (ie, the intradermal injection of both serum and saline induced a wheal-and-flare reaction). None of 20 (0%) atopic controls was positive on ASST. Intradermal injection of autologous plasma did not elicit any wheal-and-flare reaction in 3 of 4 ASST-positive patients or induced a markedly less intense reaction than autologous serum (*n* = 1).

In Vitro Tests

Sera from 4 of 14 (29%) MNS patients versus 0 of 4 (0%) SNS subjects (*P* = NS) induced significant histamine release from basophils of normal donors (Table 3). Serum from one MNS patient who did not induce histamine release from normal donor basophils induced significant histamine release from her own cultured basophils; by contrast, sera from the other four MNS patients did not induce histamine release from autologous basophils (whereas two of them did on donor basophils).

DISCUSSION

The mechanisms underlying pseudoallergic drug-induced adverse reactions have long been a matter of debate. The only certainty has always been the absence of any detectable immunologic mechanism. Multiple drug allergy reactions are typical examples of pseudoallergic reaction. This study clearly demonstrated that patients with multiple drug reactivity are characterized by the presence of circulating histamine-releasing factors. More than 90% of patients with a history of MNS included in this study showed an unequivocal wheal-and-flare reaction upon injection of autologous serum. This proportion is even greater than that observed in patients with chronic idiopathic urticaria (approximately 60%), a condition frequently associated with multiple NSAID intolerance. Thus, autoreactivity seems to be very common in clinical conditions characterized by urticaria after the ingestion of several chemically unrelated drugs, and might represent a common pathogenic background for these adverse drug reactions. One hypothesis is that offending drugs may somehow enhance or facilitate the activity of circulating histamine-releasing factors leading to acute urticaria episodes, whereas such factors alone might not be sufficiently efficient to do this. Interestingly, also approximately one-third of our controls with single NSAID intolerance were positive on ASST. A followup study is in progress to detect whether ASST-positive controls show a propensity to react against other NSAIDs. The specificity of ASST was confirmed by its negativity in 20 atopic patients without a history of drug allergy. This finding seems to rule out a nonspecific role of bradykinin or C5a generated during the clotting process.²⁵ The nature of circulating histamine-releasing factors is still unclear. The fact that only a minority of sera from ASST+ subjects were able to induce histamine release in vitro from normal as well as autologous basophils suggests that these factors might not differ from those involved in most patients

Table 3. In Vivo and In Vitro Histamine-Releasing Activity of Sera from Patients and Controls

No.	Patients			Controls	
	ASST	HRA	Auto-HRA	ASST	HRA
1	4+	Neg	13,2	4+	Neg
2	2+	12,9	Neg	Neg	Neg
3	4+	Neg	Neg	3+	Neg
4	4+	6,8	Neg	Neg	
5	4+	Neg		Neg	
6	4+	Neg		3+	Neg
7	Neg	Neg		Neg	
8	DG			Neg	
9	2+			Neg	
10	4+			Neg	
11	3+	Neg		Neg	
12	4+	Neg		Neg	
13	2+	8,1	Neg	3+	Neg
14	3+	Neg		3+	
15	4+				
16	2+				
17	3+	16,9			
18	2+				
19	4+				
20	2+	Neg			
21	3+	Neg			
22	3+	Neg			

ASST = Autologous serum skin test.

HRA = Histamine release assay.

Auto-HRA = with autologous basophils.

DG = Dermatographism.

with chronic urticaria.²² The different findings using basophils from normal donors or from patients should reflect the well known variability in releasability between basophils from different subjects.

Theoretically, the autoreactivity observed in most patients might represent a pathogenic mechanism for pseudoallergic reactions induced by drugs other than NSAIDs. Although further studies on larger populations are needed to test this hypothesis, it is interesting to observe that a significant proportion of patients had a history of urticaria also after taking antibacterial drugs, a vasoactive blueberry extract (Tegens, Synthelabo, Bagneux, France), nomegestrol, acetylcysteine, or metisoprinole, an antiviral agent (Table 1). Our findings represent an indirect confirmation of studies reporting an association between intolerance to NSAID and to antibacterial drugs.^{2,26-28} In the presence of an underlying condition facilitating nonspecific pseudoallergic drug

reactions, a history of previous drug allergy would certainly represent a risk factor for intolerance to other compounds. Obviously, several aspects remain elusive. For instance, the reason why only certain drugs seem able to induce histamine release from basophils and/or mast cells in single patients, or the interactions occurring between offending drugs (or some of their metabolic breakdown products), histamine-releasing factors, and mast cells and/or basophils. Another interesting question is: why do patients showing a strongly positive ASST not have chronic idiopathic urticaria? As suggested before, one reasonable hypothesis is that, at least in some cases, circulating histamine-releasing factors themselves may not induce sufficient histamine release to cause clinically relevant, spontaneous urticaria; in these subjects a further nonspecific trigger (the offending drug) would be needed to cause a massive histamine release from both mast cells and ba-

sophils. Studies are in progress to try to clarify at least in part these points.

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