Multiple Drug Allergy in Patients with Cutaneous Adverse Drug Reactions Diagnosed by *In Vitro* Drug-Induced Interferon-Gamma Release*

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Abstract

Background: Multiple drug allergy syndrome is a rarely reported clinical condition characterized by an adverse reaction to more than one different class of pharmacologically and structurally unrelated drugs. The pathogenesis may involve immediate-type or delayed-type hypersensitivity.

Objectives: To further characterize patients with MDA in terms of the type of CADR, drug intake and clinical drug suspicion.

Methods: The study group comprised 12 patients (6 males, 6 females) with CADRs showing *in vitro* drug-induced IFNγ release for multiple drugs, suggesting the presence of MDA. The diagnostic role of *in vitro* IFNγ release in identifying the culprit drugs was evaluated in terms of clinical data and the results of *in vivo* tests (withdrawal and/or challenge tests) with the offending drugs.

Results: Clinical relevance was attributed to *in vitro* drug-induced IFNγ release towards multiple drugs in this series of 12 patients with a variety of CADRs, implying MDA. The results of *in vivo* tests for the offending drugs confirmed the diagnosis. The main causative agents responsible were antibiotics and non-steroidal anti-inflammatory drugs.

Conclusions: The study further supports the role of a T cell-mediated mechanism in the pathogenesis of MDA. The *in vitro* drug-induced IFNγ release test may serve as a laboratory tool to identify the culprit drugs associated with this allergy.

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Cutaneous adverse drug reactions are attributed usually to the intake of a single culprit drug, although many patients report the consumption of multiple medications. There are only a small number of reports on multiple drug allergy syndrome (also known as multiple drug hypersensitivity or multiple drug intolerance syndrome), a clinical condition characterized by an adverse reaction against more than one different class of pharmacologically and structurally unrelated drugs (antibiotics or non-antibiotics) [1-12]. MDA has been associated mainly with antibiotics (multiple antibiotic syndrome) and the diagnosis is based mainly on the patient's history. Possible mechanisms that have been suggested include immediate-type hypersensitivity with the presence of circulating histamine-releasing factors [3,9,12] and delayed-type hypersensitivity to the responsible drugs via distinct T cell populations [4,8,10]. The entity of MDA should be elucidated further and safe alternative drugs should be identified for patients with this syndrome [6,11].

We present a case series of patients with MDA manifested by CADRs. Following previous studies, which implied a diagnostic role for *in vitro* drug-induced interferon-gamma release in the identification of the offending drugs in patients with CADRs [13-21], the diagnosis of MDA in the present series was based on *in vitro* drug-induced IFNγ release to multiple drugs.

Patients and Methods

The *in vitro* drug-induced IFNγ release test, which we use as a diagnostic test in patients with CADRs [20], detected a subgroup of 15 patients with *in vitro* drug-induced IFNγ release for multiple drugs, suggesting MDA. In 12 of the patients information on *in vivo* tests (withdrawal and/or challenge tests) with the offending drugs was available, based on medical files and/or medical history. A positive withdrawal test was defined as drug withdrawal followed by improvement or resolution of the reaction. A positive challenge test was defined as a challenge with the drug followed by aggravation or reappearance of the reaction.

The aim of this patient series presentation is to further characterize patients with MDA in terms of the type of CADR, drug intake and clinical drug suspicion (high, possible, low), which was determined based on the timing of drug exposure (latent period), guide tables and a literature search.

In all 12 patients an *in vitro* drug-induced IFNγ release test was performed after the acute stage of the cutaneous adverse reaction (mean time 124 days). The IFNγ test technique has been

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MDA = multiple drug allergy
CADR = cutaneous adverse drug reaction
IFNγ = interferon-gamma

described previously [15,20]. In brief, it involves incubation (24 hours, 37°C, 5% CO_2) of peripheral blood lymphocytes ($2x10^6$/ml) in M-199 medium containing fetal calf serum (5%), PHA-P (200 µg/ml), and glutamine (2 mM), with or without the tested drugs (parent drug compounds), dissolved in the appropriate solvent. Following incubation the supernatants are collected by centrifugation (4°C). The human IFNγ level (pg/ml) in culture supernatants is determined with a commercial enzyme-linked immunosorbent essay kit (Quantikine R&D Systems, Minneapolis, MN, USA). For each drug the relative percentage increase in IFNγ release, with and without the tested drug, is calculated. A threshold level is defined as the mean relative increase of IFNγ in controls + 2 standard deviations. Threshold levels are determined for a general pool of drugs as well as for specific pools of drugs (non-steroidal anti-inflammatory drugs, paracetamol, and others). A positive IFNγ release test is defined as a relative increase of IFNγ higher than the threshold level.

The mean IFNγ release for drugs taken by the 12 patients was compared to the mean IFNγ release for drugs taken by 11 controls, individuals exposed to two or more drugs who did not develop an adverse reaction. The agreement between the *in vitro* IFNγ release tests and the *in vivo* tests (withdrawal and/or challenge tests) performed in the patients was analyzed.

Results

This case series of 12 patients with suspected MDA comprised six males and six females with a mean age of 49.2 years (range 5–84). They were exposed to 42 drugs, 2–5 per patient. The clinical and laboratory data of the patients are presented in Table 1. The patients manifested a variety of CADRs including vasculitis (3 patients), urticaria (2 patients), exanthematous eruption (2 patients), and other rashes (5 patients) such as toxic epidermal necrolysis, erythema multiforme-like eruption, fixed drug eruption, Sweet's syndrome and psoriasiform eruption.

The mean IFNγ release recorded for the 42 drugs taken by the 12 patients (101.8 ± 94.8) was significantly higher ($P < 0.0001$) than the mean IFNγ release (26.3 ± 25.5) recorded for drugs taken by the 11 controls (6 males, 5 females, mean age 55.8 years, range 10–78 years) who were exposed to a total of 32 drugs (2–4 per patient) without developing adverse drug reactions.

Based on the threshold levels cited above, the IFNγ release test was interpreted as positive in 32 of 42 drugs (76.2%) taken by the patients. Positive IFNγ release tests were recorded for two drugs, three drugs, and four drugs. Drug categories with positive IFNγ test results (in order of frequency) were: NSAIDs (8 patients), antibiotics (8 patients), analgesics (7 patients), cardiovascular drugs (5 patients), and others (4 patients). Common drug combinations with positive

Table 1. Clinical and laboratory data in 12 patients with multiple drug allergy

Patient	Age/ Gender	CADR	Drug intake	Clinical suspicion	IFNγ test	Challenge test	Withdrawal test
1	59/M	Vasculitis	Acetylsalicylic a.	High	+		
			Atenolol	High	+		
			Resprim®	High	+		+
			Cefuroxime	High	+		+
2	60/M	Vasculitis	Acetylsalicylic a.	High	+		+
			Atenolol	High	+		+
			Rokal ®	High	–	+	
			Pravastatin	Low	–	–	
			Codeine phos.	Low	–		+
3	51/M	Vasculitis	Atenolol	High	+		+
			Amlodipine	High	+	+	
			Loratadine	Low	+		+
4	31/F	Urticaria	Minocycline	High	+		+
			Rafatricin®	Low	+		
5	68/F	Urticaria	Amoxycillin	High	+		+
			Augmentin®	High	+		+
			Naproxen	High	+		+
6	53/F	Exanthematous eruption	Clindamycin	High	+		+
			Amoxycillin	Low	+	–	
			Ibuprofen	Low	+	+	
			Paracetamol	Low	+	–	
			Lignocaine HCl	Low	–	–	
7	84/M	Exanthematous eruption	Acetylsalicylic a.	High	+		+
			Diclofenac sod.	Possible	+		+
			Atenolol	Low	–	–	
			Warfarin sodium	Low	–	–	
8	5/M	Toxic epidermal necrolysis	Paracetamol	High	+		
			Histafed®	High	+		
			Ibuprofen	Low	+	–	
			Dipyrone	Low	+	–	
9	29/F	Erythema multiforme-like	Lignocaine HCl	High	+		+
			Fentanyl	High	+		+
			Bupivacaine	Low	–	–	
10	43/F	Fixed drug eruption	Naproxen	High	+		+
			Paracetamol	High	+		+
11	70/M	Psoriasiform eruption	Atenolol	High	–	–	
			Propranolol	High	+		+
			Acetylsalicylic a.	Possible	+	–	
			Felodipine	Low	–	–	
12	38/F	Sweet's syndrome	Amoxycillin	High	–	–	
			Paracetamol	High	+	+	
			Dipyrone	High	+	+	
Total			42 drugs	26 High 2 Possible 14 Low	32 + 10 –	5 + 13 –	19 +

M = male, F = female, CADR= cutaneous adverse drug reaction, Rafatricin® = tyrothricin + benzocaine, Resprim® = trimethoprim + sulfamethoxazole, Rokal® = acetylsalicylic a + caffeine + codein phosphate, Histafed® = triprolidine + pseudoephedrine, Augmentin® = amoxycillin + clavulanic acid

NSAIDs = non-steroidal anti-inflammatory drugs

IFNγ tests were: NSAIDs and antibiotics (patients 1, 5 and 6), NSAIDs and beta blockers (patients 1, 2 and 11), and NSAIDs and analgesics (patients 6, 8 and 10).

The rate of positive IFNγ responses within the three groups of drug suspicion was 23/26 (88.5%) for drugs with a high level of suspicion, 2/2 (100%) for drugs with possible suspicion, and 7/14 (50%) for drugs with low suspicion. The occurrence of positive IFNγ responses for the high suspicion drugs (23/26, 88.5%) was significantly higher ($P = 0.018$, two-tailed Fisher's exact test) than that recorded for the low suspicion drugs (7/14, 50%).

A total of 37 *in vivo* tests (18 challenge tests, 19 withdrawal tests) were conducted. Positive *in vivo* tests were recorded for 20/26 (76.9%) of the high suspicion drugs, for 1/2 (50%) of the possible drugs, and for 3/14 (21.4%) of the low suspicion drugs. Positive *in vivo* tests for the high suspicion drugs (20/26, 76.9%) were significantly higher ($P < 0.0023$, Yates corrected) than for the low suspicion drugs (3/14, 21.4%). A comparison between the results of *in vitro* IFNγ release tests and the results of *in vivo* tests revealed 85.7% agreement, kappa 0.560, which implies an intermediate to good degree of agreement.

A comparison between the results of the 18 challenge tests (considered the "gold standard" in adverse drug reactions) and the results of the 18 IFNγ release tests revealed the following values: sensitivity 80%, specificity 62%, positive predictive value 44%, negative predictive value 89%.

Discussion

The mechanisms underlying multiple drug reactivity are still unclear. Based on the wheal-and-flare reaction and the *in vitro* basophil histamine release assay, the involvement of immediate-type hypersensitivity, with the presence of circulating histamine-releasing factors, is possible [3,9,12]. Another possible mechanism is delayed-type hypersensitivity driven by distinct T cell populations (CD4+ and CD8+ cells), showing a heterogeneous pattern of cytokine production [4,8,10].

Drug-specific T cells are also involved in distinct clinical manifestations of CADRs, and may orchestrate the inflammatory skin reaction through the release and induction of different cytokines [22]. Furthermore, the drug-induced release of the Th1 cytokine IFNγ from patients' peripheral blood lymphocytes following *in vitro* challenge with drugs points to a drug-specific cellular immune response (delayed-type hypersensitivity or cell-mediated immunity) and may facilitate the identification of the offending drugs in CADR [13-21].

In the present series of 12 patients with CADRs, MDA was diagnosed by the *in vitro* drug-induced release of the Th1-type cytokine IFNγ towards multiple drugs. Clinical relevance was attributed to *in vitro* drug-induced IFNγ release towards multiple drugs based on the following data: a) the mean IFNγ release in the patients was significantly higher than in controls, b) the distribution of positive IFNγ release tests was significantly higher for high suspicion compared to low suspicion drugs, and c) a good to intermediate degree of agreement was found between the *in vitro* IFNγ release tests and the *in vivo* tests. The sensitivity, specificity, positive predictive value and negative predictive value that were obtained for the IFNγ release test may reflect its usefulness as a diagnostic test in MDA. However, in the present study the values obtained may have been influenced by the small number of patients.

The results of the present study are consistent with a recent study of seven patients in whom multiple drug sensitivity was diagnosed *in vitro* by the lymphocyte transformation test and confirmed *in vivo* by patch tests towards the offending drugs [4]. Both studies imply that a T cell-mediated mechanism plays a role in MDA.

The relevant medical literature contains reports of a variety of clinical manifestations associated with this syndrome, including acute urticaria and/or angioedema (the majority of cases), anaphylaxis, serum sickness-like reaction, erythema multiforme/Stevens-Johnson syndrome, immune cytopenia [1,5,7,9], and a maculopapular rash [4,10]. Acute generalized exanthematous pustulosis, fixed drug eruption, and erythema multiforme have also been associated with polysensitivity to drugs [16,23,24]. In the present series of MDA the clinical manifestations were cutaneous adverse reactions including urticaria, toxic epidermal necrolysis, erythema multiforme-like eruption and fixed drug eruption, manifestations that were previously associated with MDA or polysensitivity. However, to the best of our knowledge, vasculitis, which was recorded in three of the patients, has not been reported as a manifestation of MDA.

Antibiotics are often involved in this syndrome. Sensitization or intolerance to other drugs, such as antiepileptics, local anesthetics and NSAIDs has also been reported in MDA [4,10,12]. NSAIDs have been implicated as a risk factor for multiple antibiotic sensitivity [1,6]. In the present series the main culprit drugs, based on positive IFNγ test results, were antibiotics (8 patients) and NSAIDs (8 patients). The simultaneous occurrence of positive IFNγ tests for NSAIDs and antibiotics, observed in 3 of 12 patients, may support the role of NSAIDs as a risk factor for multiple antibiotic sensitivity.

In the present series, evaluation of the culprit drugs in terms of the type of CADR was consistent with reported associations in the literature, such as vasculitis associated with beta-blockers and NSAIDs, urticaria associated with antibiotics, exanthematous eruption associated with antibiotics, fixed drug eruption associated with naproxen and paracetamol, and psoriasiform eruption associated with propranolol and acetylsalicylic acid [25], suggesting causality.

In light of the above, it appears that in the present series of patients with CADRs, the positive IFNγ responses recorded for multiple drugs imply MDA rather than false positive IFNγ test results. Female predominance, which has been reported to be a risk factor for multiple antibiotic sensitivity [1,6], was not seen in the present series probably due to the small sample size or the profile of the offending drugs.

In conclusion, the present study supports the concept that some individuals are likely to develop an adverse drug reaction to more than one drug, the so-called MDA syndrome. I*n vitro* drug-induced IFNγ release may serve as a laboratory tool for the identification of the drugs responsible for MDA.

References

1. Asero R. Detection of patients with multiple drug allergy syndrome by elective tolerance tests. *Ann Allergy Asthma Immunol* 1998;80:185–8.
2. Asero R. Multiple drug allergy syndrome: a distinct clinical entity. *Curr Allergy Rep* 2001;1:18–22.
3. Asero R, Tedeschi A, Lorini M, Caldironi G, Barocci F. Sera from patients with multiple drug allergy syndrome contain circulating histamine-releasing factors. *Int Arch Allergy Immunol* 2003; 131:195–200.
4. Gex-Collet C, Helbling A, Pichler WJ. Multiple drug hypersensitivity – proof of multiple drug hypersensitivity by patch and lymphocyte transformation tests. *J Investig Allergol Clin Immunol* 2005; 15:293–6.
5. Khoury L, Warrington R. The multiple drug allergy syndrome: a matched-control retrospective study in patients allergic to penicillin. *J Allergy Clin Immunol* 1996;98:462–4.
6. Nettis E, Colanardi MC, Paola RD, Ferrannini A, Tursi A. Tolerance test in patients with multiple drug allergy syndrome. *Immunopharmacol Immunotoxicol* 2001;23:617–26.
7. Park J, Matsui D, Rieder MJ. Multiple antibiotic sensitivity syndrome in children. *Can J Clin Pharmacol* 2000;7:38–41.
8. Scala E, Giani M, Pastore S, et al. Distinct delayed T-cell response to beta-methasone and penicillin-G in the same patient. *Allergy* 2003;58:439–44.
9. Scala E, Giani M, Pirrotta L, Guerra EC, De Pita O, Puddu P. Multiple drug allergy syndrome: severe anaphylactic reaction due to topical rifamycin SV in a patient with hypersensitivity to ciprofloxacin. *Int J Dermatol* 2001;40:603–4.
10. Scala E, Giani M, Pirrotta L, et al. Simultaneous [correction of Spontaneous] allergy to ampicillin and local anesthetics. *Allergy* 2001;56:454–5.
11. Schiavino D, Nucera E, Roncallo C, et al. Multiple-drug intolerance syndrome: clinical findings and usefulness of challenge tests. *Ann Allergy Asthma Immunol* 2007;99(2):136–42.
12. Tedeschi A, Lorini M, Suli C, Cugno M. Detection of serum histamine-releasing factors in a patient with idiopathic anaphylaxis and multiple drug allergy syndrome. *J Investig Allergol Clin Immunol* 2007;17(2):122–5.
13. Koga T, Imayama S, Hori Y. In vitro release of interferon-γ by peripheral blood mononuclear cells of a patient with carbamazepine-induced allergic drug eruption in response to stimulation with carbamazepine. *Contact Dermatitis* 1995;32:181–2.
14. Halevy S, Cohen AD, Livni E. The diagnostic role of the *in vitro* interferon-gamma release test in Stevens-Johnson syndrome. *Int J Dermatol* 1999;38:835–40.
15. Livni E, Lapidoth M, Halevy S. In vitro release of interferon-gamma and macrophage migration inhibition factor in drug-induced urticaria and angioedema. *Acta Derm Venereol Suppl (Stockh)* 1999;79: 18–22.
16. Halevy S, Cohen AD, Livni E. Acute generalized exanthematous pustulosis associated with polysensitivity to paracetamol and bromhexine. The diagnostic role of in vitro interferon-γ release test. *Clin Exp Dermatol* 2000;25:652–4.
17. Halevy S, Cohen AD, Grossman N. In vitro interferon-gamma release – a laboratory diagnosis of cutaneous adverse drug reactions. *Harefuah* 2001;140:121–4, 90 (Hebrew).
18. Halevy S, Cohen AD, Lunenfeld E, Grossman N. Autoimmune progesterone dermatitis manifested as erythema annulare centrifugum: confirmation of progesterone sensitivity by in vitro interferon-gamma release. *J Am Acad Dermatol* 2002;47:311–13.
19. Halevy S, Gold I, Cohen AD, Grossman N. In vitro interferon-gamma release test in the diagnosis of drug-induced erythema nodosum. *IMAJ* 2004;6:59–60.
20. Halevy S, Cohen AD, Grossman N. Clinical implications of in vitro drug-induced interferon gamma release from peripheral blood lymphocytes in cutaneous adverse drug reactions. *J Am Acad Dermatol* 2005;52:254–61.
21. Goldberg I, Gilburd B, Kravitz MS, et al. A novel system to diagnose cutaneous adverse drug reactions employing the cells-can – comparison with histamine releasing test and Inf-gamma Releasing Test. *Clin Dev Immunol* 2005;12:85–90.
22. Pichler W, Yawalkar N, Schmid S, Helbling A. Pathogenesis of drug-induced exanthems. *Allergy* 2002;57:884–93.
23. Ozkaya E. Polysensitivity in fixed drug eruption due to a novel drug combination-independent lesions due to piroxicam and cotrimoxazole. *Eur J Dermatol* 2006;16:591–2.
24. Isik SR, Karakaya G, Erkin G, Kalyoncu AF. Multidrug-induced erythema multiforme. *J Investig Allergol Clin Immunol* 2007;17:196–8.
25. Revuz J, Valeyrie-Allanore L. Drug reactions. In: Bolognia J, Jorizzo J, Rapini R, eds. Dermatology. 2nd edn. Spain: Mosby, Elsevier, 2008.

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