

The use of skin testing in the investigation of cutaneous adverse drug reactions

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Summary

Skin testing with the suspected compound has been reported to be helpful in determining the cause of cutaneous adverse drug reactions (ADRs), but the value and specificity of these tests need to be determined. In this study, 72 patients with presumed drug eruptions (27 maculopapular, 18 urticarial, seven erythrodermic, nine eczematous, four photosensitivity, three fixed drug eruptions, three with pruritus and one with acute generalized exanthematous pustulosis) were assessed. All had drug patch tests; 46 also had prick tests and 30 had intradermal tests (performed on hospitalized patients using a sterile solution of the suspected drug, diluted sequentially) with immediate and delayed readings. Among these patients, 52 (72%) had a positive skin test reaction, 43%, 24% and 67% in patch, prick and intradermal skin tests, respectively. The results of skin tests varied with the drug tested and with the clinical type of cutaneous ADR, as a significantly higher number of positive patch tests was observed in maculopapular rashes than in urticarial reactions ($P = 0.001$). This study supports the value of careful sequential drug skin testing in establishing the cause of cutaneous ADR. Guidelines are proposed for performing these tests, and these include the use of appropriate negative control patients to avoid false-positive results.

The skin is affected in 25–30% of adverse drug reactions (ADRs).¹ Different mechanisms are responsible for such cutaneous drug reactions, but some ADRs are thought to be of immunological origin.^{1–3} Skin testing with the suspected drug may be helpful in determining the cause of a cutaneous ADR. It may include drug patch tests (or photopatch tests), prick tests and/or intradermal tests (IDRs).

In immediate Ig E-mediated drug reactions, rules defining the procedures for prick tests and IDRs have been proposed in previous studies,⁴ but care must be taken in reading not only the immediate but also the delayed reactions of these skin tests. Cutaneous ADRs thought to be caused by a delayed cell-mediated immune reaction,^{1–3} such as maculopapular rashes (MPRs), fixed drug eruptions (FDEs), lichenoid or eczematous reactions, are rarely evaluated by means of these tests. However, as we and others have shown, patch tests can be helpful for the aetiological diagnosis.^{5–15} Osawa *et al.*¹⁴ have reported positive drug patch test results in 31.5% of cutaneous ADRs of non-immediate type. More specifically, the positivity rates

reported in the literature vary between 17% and 35% in investigated cutaneous ADRs caused by penicillin derivatives.^{8,11} In spite of the interest of such investigations, the literature lacks prospective studies determining the value and specificity of drug skin tests in cases of cutaneous ADRs. Guidelines are also lacking concerning the procedure to use for each drug and the various clinical forms of cutaneous ADRs.

This paper reports a study in which the clinical features of the cutaneous ADRs, the chronology of drug intakes and the imputability of each drug (according to the criteria proposed by Moore *et al.*¹⁶) were assessed for 72 patients. Drug patch tests, often with associated prick tests and IDRs, were performed 6 weeks after the onset of the cutaneous ADRs. Immediate and delayed readings were evaluated for each series of tests.

Patients and methods

Patients

This study received the approval of the medical ethics committee of the University Hospital of Nancy (France). For each of the 310 patients enrolled, clinical features of

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Table 1. Criteria for inclusion of patients and some details of investigations undertaken

Clinical features of the cutaneous adverse drug reaction (ADR) were observed by at least one of the coauthors, described, photographed and classified
Skin biopsy with histological study was performed at the onset of the cutaneous ADR
Chronology of drug intakes and imputability for each drug taken before or during the onset of dermatosis could be determined according to criteria proposed by Moore <i>et al.</i> ¹⁶
One drug was identified as being very likely to be the cause of the eruption at its onset
The following investigations were performed: full blood count, platelet count, erythrocyte sedimentation rate; serum creatinine, transaminases, alkaline phosphatase, bilirubin; immunoglobulin levels, complement fraction assays, circulating immune complex assays, lymphocyte subset assessment; tests for <i>Herpes hominis</i> types I and II, <i>Mycoplasma</i> , cytomegalovirus, <i>Rickettsia</i> , Epstein–Barr virus, <i>Haemophilus influenzae</i> b, parvovirus B19, Coxsackie virus, enterovirus serologies; antistreptolysins, antistreptokinases, antistreptodornase assays
Patients had given informed consent for drug patch tests, 6–10 weeks after the onset of the cutaneous ADRs
Drug patch tests could be performed at least 1 month after the discontinuation of local or systemic corticosteroid therapy
Patients were off antihistamines or beta-blockers when the skin tests were performed

the dermatosis were classified. Imputability for each drug taken before or during the onset of dermatosis was determined, according to criteria proposed by Moore *et al.*¹⁶ A total of 72 patients fulfilled the inclusion criteria (Table 1). All of the 72 patients included had a very likely intrinsic imputability of one drug at the onset of dermatosis and had drug patch tests 6 weeks after the onset of cutaneous ADR. Thirty-one of the 72

patients had had at least two episodes of similar cutaneous ADR after taking the culprit drug again (accidental rechallenge).

Clinical aspects of these 72 patients (24 men and 48 women; mean age 52.3 years, SD 21.4 years) were as follows: 21 MPRs, six maculopapular and purpuric rashes, 14 urticarias, four urticarias with angioedema, seven erythrodermas (exfoliative dermatitis), eight

Table 2. Skin test results in patients with cutaneous adverse drug reactions induced by beta-lactam antibiotics

Patient number	Presentation	Patch test	Prick test		Intradermal testing		Reintroduced	Drug
			Immediate	Delayed	Immediate	Delayed		
1	MPR	–	–	–	–	–	0	Amoxycillin
2	MPR	–	–	–	–	–	0	Amoxycillin
3	MPR	++/++	–	+	–	+	0	Amoxycillin
4	MPR	++/++	–	–	–	+	+	Amoxycillin
5	MPR	++/++	–	+	–	+	+	Amoxycillin
6	MPR	0/+	ND	–	ND	–	0	Amoxycillin
7	MPR	++/++	–	+	–	+	+	Amoxycillin
8	MPR	–	–	–	+	–	0	Amoxycillin
9	MPR + pur	+/+	–	–	–	+	0	Amoxycillin
10	MPR + pur	–	–	–	–	+	0	Amoxycillin
11	Urticaria	–	–	–	–	–	0	Amoxycillin
12	Urticaria	–	–	–	+	+	0	Amoxycillin
13	Urticaria	+/++	–	–	ND	–	0	Amoxycillin
14	Urticaria	–	+	–	ND	–	+	Amoxycillin
15	Urticaria	–	–	–	+	–	0	Amoxycillin
16	Erythro D	–	–	–	–	+	+	Amoxycillin
17	G eczema	–	–	–	–	+48 h	0 ^b	Amoxycillin
18	G eczema	0/+	ND	–	ND	–	0	Penicillin G
19	Urticaria	–	+	–	ND	–	+	Cefapirin
20	FDE	–	ND	–	ND	–	+	Cefpodoxime
21	AGEP	+/+	ND	–	ND	–	0	Cloxacillin

MPR, maculopapular rash; MPR + pur, MPR with purpura; G eczema, generalized eczema; L eczema, localized eczema; Erythro D, erythroderma; FDE, fixed drug eruption; AGEP, acute generalized exanthematous pustulosis; photoS, photosensitivity; urt + AO, urticaria with angioedema. Results of patch tests are given on day 2 and 4 readings, according to International Contact Dermatitis Research Group criteria for patch test reading.¹⁷ ND, not done; ^apositive photopatch test; ^breactivation of the cutaneous ADR after skin testing.

Table 3. Skin test results in patients with cutaneous adverse drug reactions induced by non-beta-lactam antibiotics (see legend to Table 2 for abbreviations)

Patient number	Presentation	Patch test	Prick test		Intradermal testing		Reintroduced	Drug
			Immediate	Delayed	Immediate	Delayed		
22	Urticaria	–	–	–	–	+	0	Erythromycin
23	MPR	–	+	–	ND		0	Erythromycin
24	Urticaria	–	–	–	ND		+	Josamycin
25	Urticaria	–	+	–	ND		0	Spiramycin
26	urt + AO	–	–	–	–	–	+	Spiramycin
27	MPR	+/+	ND		ND		0	Pristinamycin
28	MPR	++/++	ND		ND		0	Pristinamycin
29	MPR	0/+	ND		ND		0	Pristinamycin
30	MPR	+/+	ND		ND		0	Pristinamycin
31	MPR	0/+	ND		ND		0	Pristinamycin
32	Erythro D	–	–	–	ND		0	Pristinamycin
33	Erythro D	+/+	ND		ND		0	Pristinamycin
34	Erythro D	+/+	ND		ND		0	Pristinamycin
35	Erythro D	++/++	ND		ND		0	Pristinamycin
36	FDE	–	–	–	–	–	+	Doxycycline
37	MPR	–	–	–	ND		+	Co-trimoxazole
38	Urticaria	–	–	–	+	–	+ ^b	Co-trimoxazole
39	MPR + pur	–	–	–	ND		+	Norfloxacin
40	MPR + pur	–	–	–	ND		+	Pipemidic acid
41	MPR	–	–	–	–	–	+	Isoniazid
42	MPR	+/+	ND		ND		0 ^b	Aciclovir

generalized eczemas, one localized eczema, four photo-sensitivity eczematous reactions, three FDEs, three pruritus and one acute generalized exanthematous pustulosis (AGEP) (Tables 2–4). The drugs involved were anti-infectious agents in 42 cases: beta-lactam drugs in 21 cases (Table 2); macrolides and related drugs in 14 cases; co-trimoxazole in two cases; quinolones in two cases; and doxycycline, aciclovir and isoniazid in one case each (Table 3). The various drugs responsible for cutaneous ADR in the other 30 patients are reported in Table 4.

Patch tests

Patch tests were performed on the upper back of all 72 patients using Finn chambers on Scanpor tape® (Epi-test, Tuusula, Finland). In all patients, patch tests of the European standard series were used, and the drug responsible for the cutaneous ADR was tested with the commercialized form used by the patient. In all, 37 different drugs were investigated. Pills in powder form were tested diluted to 30% in white petrolatum (pet.) and diluted to 30% in water. The content of capsules was tested diluted to 30% in pet. and diluted to 30% in water. The gel jacket portion of capsules was moistened

and tested. Liquid preparations were tested both neat and diluted 1 : 1 in water. For 27 of the drugs involved, the pure drug was also tested diluted to 30% in pet. and to 30% in water. Additionally, the excipients and preservatives contained in the commercialized drugs were tested (Table 5). Patch test reactions were read at 20 min and 2, 3 and 4 days. The results of patch testing were reported according to the International Contact Dermatitis Research Group criteria for patch test reading.¹⁷

Prick tests

In 46 cases, prick tests were performed on the volar forearm skin with the commercialized form of the drug thought to be the agent of the cutaneous ADR. Whenever possible, both the pure drug and excipients were tested (in the form used for patch testing). If urticaria had developed in the patient, the drug was tested with the same sequential solutions as those used for IDR at 10^{-3} , 10^{-2} , 10^{-1} and then pure.

Except for investigations performed with amoxycillin and in one other case (no. 68), prick tests were performed only when drug patch tests had produced negative results. In two cases (nos 20 and 58), prick

Table 4. Skin test results in patients with cutaneous adverse drug reactions induced by non-antibiotic drugs (see legend to Table 2 for abbreviations)

Patient number	Presentation	Patch test	Prick test		Intradermal testing		Reintroduced	Drug
			Immediate	Delayed	Immediate	Delayed		
43	G eczema	–	–	–	–	–	0	Amiodarone
44	Urticaria	–	–	–	–	–	+	Articaine
45	Urticaria	–	–	–	–	–	+	Articaine
46	Erythro D	0/+	ND		ND		0	Captopril
47	MPR	–	–	–	–	–	0	Carbamazepine
48	MPR + pur	+/+	ND		ND		0	Carbamazepine
49	MPR + pur	–	–	–	+	–	0 ^b	Cyamemazine
50	MPR	0/++	ND		ND		0	Diltiazem
51	G eczema	–	–	–	–	+	0	Enoxoparin
52	G eczema	+/+	ND		ND		+	Enoxoparin
53	L eczema	–	–	–	–	+	+	Enoxoparin
54	G eczema	–	–	–	+	+	+	Oestradiol
55 ^a	PhotoS	–	ND		ND		0	Fluvoxamine
56	urt + AO	+/+	ND		ND		0	Fusafungine
57	Pruritus	–	–	–	ND		+	Glibenclamide
58	Pruritus	–	ND		ND		+	Heparin
59 ^a	PhotoS	–	ND		ND		0	Hydroquinidine
60 ^a	PhotoS	–	ND		ND		0	Hydroquinidine
61	Erythro D	+/+	ND		ND		0 ^b	Hydroxyzine
62 ^a	PhotoS	–	ND		ND		+	Methoxsalen
63	Urticaria	–	+	–	ND		+	Paracetamol
64	urt + AO	–	+	–	ND		+ ^b	Pholcodine
65	FDE	–	–	–	–	+	+	Piroxicam
66	urt + AO	–	–	–	+	+	+	Piroxicam
67	Urticaria	–	–	–	+	+	+	Piroxicam
68	MPR	++/++	–	+	ND		+ ^b	Pseudoephedrine
69	G eczema	–	–	–	ND		+	Simvastatine
70	MPR	+/+	ND		ND		0	Tetrazepam
71	Pruritus	–	+	–	ND		+	Tetrazepam
72	G eczema	++/++	ND		ND		0 ^b	Triamcinolone

tests were not performed, because the patients had refused. Reactions were considered positive when a weal with a diameter larger than that of the negative control (0.9% saline) was present 20 min later. Positive controls were performed with codeine phosphate at 9%. In all cases, a delayed reading was performed 24 h after the initial tests.

Intradermal tests

IDRs were performed only when prick tests showed negative results 20 min after testing with the suspected drug. IDRs were never performed in patients with erythema multiforme (EM), toxic epidermal necrolysis (TEN) or leucocytoclastic vasculitis on histological examination. Because no sterile forms of josamycin, pristinamycin and pipemidic acid could be obtained for skin testing, IDRs with these drugs could not be performed. In an additional three cases (nos 21, 25 and

37), the patients refused IDRs. The pure drugs tested, vehicles, as well as the nature and the concentration of the initial dilution used are reported in Table 5.

The following guidelines were applied for IDRs.

1 Dilutions were prepared under a laminar flow no more than 2 h before administration.

2 All necessary precautions were taken in case emergency treatment should be needed. Tested patients were hospitalized for a minimum of at least 24 h after the IDRs. Intravenous glucose solution was given for at least 2 h after the last IDR when the investigation results remained negative, and for at least 6 h if the IDR had positive results. Blood pressure, pulse and peak flow were monitored regularly.

3 IDRs were performed using a sterile solution of the suspected drug, diluted sequentially (10^{-4} , 10^{-3} , 10^{-2} and 10^{-1}) in phenolated saline (0.5% phenol in 0.9% saline).

4 IDRs were performed on the extensor surface of the

Table 5. Dilutions used for skin testing. The pure tested preparations are reported as follows. If they were in powder, all these preparations were tested neat, diluted to 30% in water and in petrolatum (pet.). If they were liquid, they were tested neat and diluted to 30% in water. Intradermal tests (IDRs) were performed using the following solutions, diluted sequentially (10^{-4} , 10^{-3} , 10^{-2} , 10^{-1}) and pure. Pure preparations for patch tests (PTs) and for IDRs were used for prick tests

Aciclovir: PT = pure in powder; amiodarone: PT and IDR = 50 mg/mL in water; amoxycillin: PT = pure in powder; IDR = 1 g/2.5 mL; ampicillin: PT = pure in powder; articaine: PT and IDR = 72 mg/1.8 mL; captopril: PT = pure in powder; carbamazepine: PT = pure in powder; cefadroxil: PT = in powder from the content of commercialized capsules containing lactose, silica and magnesium stearate; IDR = 100 mg/mL; cefapirin: PT = pure in powder; IDR = 0.2 g/mL; cefpodoxime: PT = pure in powder; chlormezanone: PT = in powder in a commercialized pill containing calcium phosphate, starch, gelatine, saccharine and gelatine; cloxacillin: PT = pure in powder; co-trimoxazole: PT and IDR = 80 mg/mL in water; cyamemazine: PT and IDR = 10 mg/mL in a commercialized solution containing acetic acid, ascorbic acid and polyoxyethylene glycol in water; danazol: PT = pure in powder; diltiazem: PT = pure in powder; doxycycline: PT and IDR = 20 mg/mL in water in a commercialized solution with magnesium chlorate, polyvidone and amino-2-ethanol; enoxoparin: PT = 100 mg/mL in water; erythromycin: PT = pure in powder; IDR = specific positivity only to 0.2 mg/mL; fusafungine: PT = in powder; fluvoxamine: PT = pure in powder; glibenclamide: PT = in powder from a commercialized pill containing lactose, starch, talc, silica and magnesium stearate; heparin: PT = 25,000 IU/mL in water; hydroquinidine: PT = in powder from the content of a commercialized capsule containing starch, polyvidone, Eudragit L, methacrylic acid, talc and stearic acid; hydroxyzine: PT = at 50 mg/mL in a commercialized form diluted in sodium hydroxide and water; isoniazid: PT = in powder in a commercialized pill with lactose, starch and magnesium stearate; IDR = 100 mg/mL in a commercialized solution with methyl and propyl parahydroxybenzoates; josamycin: PT = in powder in a commercialized form containing sodium lauryl sulphate, starch and aspartam; methoxsalen: PT = in commercialized solution at 0.1 g/mL diluted in ethyl alcohol and water; norfloxacin: PT = in powder from a commercialized pill containing hydroxycellulose, magnesium stearate and titanium oxide; oestradiol: PT = pure in powder; IDR = 0.1 mg/mL after solubilization in alcohol; paracetamol (acetaminophen): PT = pure in powder; IDR = 0.1 mg/mL after solubilization in alcohol; penicillin G: PT = in powder with sodium citrate; IDR = specific positivity only to 5×10^5 U/mL; pholcodine: PT = pure in powder; pipemidic acid: PT = in powder in a commercialized form with carboxymethylcellulose, magnesium stearate, starch and talc; piroxicam: PT = pure in powder; IDR = 20 mg/mL; pristinamycin: PT = pure in powder; pseudoephedrine: PT = pure in powder; simvastatin: PT = in powder in a commercialized form with carboxymethylcellulose, starch, butylhydroxyanisole, ascorbic acid, citric acid and magnesium stearate; spiramycin: PT = pure in powder; IDR = specific positivity only to 375 IU/mL; tetrazepam: PT = pure in powder; IDR = 10 mg/mL; triamcinolone: PT = 1% in pet.

Drug preservatives colouring agents and excipients:

Alcohol 95°: PT = 70% and 10% in water; benzyl alcohol: PT = 5% in pet.; carboxymethylcellulose: PT = 5% in water; cellulose: PT = pure in powder; erythrosin: PT = 2% in pet. and 10% in water; gelatine: PT = pure in powder; lactose: PT = pure in powder and 50% in pet.; magnesium stearate: PT = pure and 10% in pet.; methyl parahydroxybenzoate: PT = 3% in pet.; IDR = 1 mg/mL (10 negative controls); neroli essential oil: PT = 10% in pet.; polyoxyethylene glycol 1540: PT = pure in powder; propylene glycol: PT = 5% in pet. and 10% in water; saccharine: PT = pure in powder; silicate (aerosil 200): PT = pure in powder; starch: PT = pure in powder and 10% in water; stearic acid: PT = 5% in pet.; talc: PT = pure in powder.

arm, with a small volume (0.04 mL) that produced a weal of 4–6 mm in diameter. A negative control was performed with phenolated saline. When read after 30 min, IDR results were considered positive if a weal of more than 10 mm in diameter was observed. The first IDR was performed with a 10^{-4} dilution. If the results were negative after 30 min, subsequent IDRs were performed with increasingly higher serial dilutions up to the pure solution, at 30-min intervals, as long as the test results of the previous dilution were negative.

5 IDR result readings were performed at 30 min, 6 h and 24 h. If a delayed positive reaction was observed, the diameter of the papule was measured. When the IDR results were negative, a telephone call to the patient 1 week later confirmed whether the results were indeed negative or not.

Preservatives, colouring agents and excipients

The method of testing these drug components is reported in Table 5.

Negative controls for immunological investigations

In order to ensure the absence of non-significant skin reactivity (irritation) at the doses used for skin tests, a population of negative control subjects was recruited (Table 6). These control subjects were patients who had demonstrated a dermatosis 6 weeks previously, while taking several drugs, including one of those implicated in this study. They were considered negative control subjects if they failed to display reactivity to one of these drugs, while another drug was confirmed to be responsible for the cutaneous ADR they had had. For example, any patient who had taken amoxycillin and paracetamol, was demonstrated to have displayed a cutaneous ADR resulting from amoxycillin and displayed negative skin tests with paracetamol was enrolled as a negative control for paracetamol. Additionally, for intradermal testing with penicillin G, erythromycin and spiramycin, the irritant concentrations were controlled and confirmed by performing IDRs in five healthy volunteers (who are all coauthors of this paper).

Table 6. Number of drug patch, prick and intradermal tests performed in negative control patients

Drug tested	Patch test	Prick test	Intradermal testing
Amoxycillin	59	42	22
Penicillin G	16	9	7
Cefapirin	6	5	4
Cefpodoxime	2	0	0
Cloxacillin	2	1	0
Erythromycin	24	7	7
Josamycin	13	6	0
Spiramycin	8	8	6
Pristinamycin	11	3	0
Doxycycline	6	2	2
Co-trimoxazole	4	3	2
Norfloxacin	7	3	0
Pipemidic acid	1	1	0
Isoniazid	1	1	1
Aciclovir	17	2	0
Amiodarone	6	3	1
Articaine	12	5	3
Captopril	7 ^a	0	0
Carbamazepine	4	2	1
Cyamemazine	4	3	0
Diltiazem	8	0	0
Enoxoparin	11	5	0
Fluvoxamine	5 photopatch	0	0
Fusafungine	7	0	0
Glibenclamide	3	1	0
Heparin	9	8	5
Hydroquinidine	3 photopatch	0	0
Hydroxyzine	8	0	0
Methoxsalen	0 photopatch	0	0
Oestradiol	6	3	3
Paracetamol	92	22	17
Pholcodine	4	3	0
Piroxicam	20	6	6
Pseudoephedrine	8	2	0
Simvastatine	2	0	0
Tetrazepam	18	7	0
Triamcinolone	10	0	0

^aOne of the negative control patients tested with captopril had a positive patch test.

Statistics

Statistical analysis, comparing the incidence of positive skin test reactions according to the initial clinical features of the cutaneous ADRs, were performed using MYOSOTIS software (Coultronics France, Margency, France) with a χ^2 test.

Results

Patient data are reported in Table 2 for beta-lactam

antibiotics, in Table 3 for other anti-infectious agents and in Table 4 for other drugs.

Patch tests with drugs

None of the patients developed immediate positive patch test reactions. Twenty-seven patients of 72 (37.5%) had positive drug patch test reactions at 2 days and/or 4 days. In 21 patients, patch test reactions were positive from readings taken on days 2 and 4. Six patients (nos 6, 18, 29, 31, 46 and 50) developed a positive patch test reaction only for the reading taken on day 4. Frequencies of positive drug patch test reaction distribution according to initial clinical features were as follows: MPRs, 14 of 21 (67%); maculopapular and purpuric rashes, two of six [therefore, in all MPRs, 16 of 27 (59%)]; urticaria, one of 14 (7%); angioedema, one of four [therefore, in urticaria and angioedema, two of 18 (11%)]; erythroderma, five of seven; eczema, three of nine; AGEF, one of one; FDEs, none of three; photosensitivity reactions, none of four; and pruritus, none of three. In patient 13, a positive patch test reaction was obtained only when amoxycillin was diluted in petrolatum, but the result was negative when the drug was tested neat or diluted in water. The three patients with FDEs had negative patch test results when tested on the pigmented site of the previous ADR.

The four patients with photosensitivity all had positive photopatch test results. During PUVA therapy with methoxsalen, prescribed for psoriasis, one of these four patients (no. 62) developed a severe generalized eczema. For the same patient, the results of a photopatch test with methoxsalen (irradiated with UVA) were strongly positive, with histological features of eczema on the skin biopsy.

Among the 72 patients undergoing patch tests, five had a slight relapse of cutaneous ADR: after a positive patch test reaction with aciclovir (no. 42), amoxycillin (no. 17), hydroxyzine (no. 61), pseudoephedrine (no. 68) or triamcinolone (no. 72).

Prick tests

Prick tests were performed on 46 patients, and the following was observed: 11 had positive reactions (24%); seven (15%) of them with only an immediate positive reaction at the 20 min reading and four (9%; with a clinical feature of MPR) with only a delayed positive reaction at the 24 h reading. Among the patients with initial clinical features of MPR, five of 17 (29%) had positive prick test reactions, one of them with only an immediate positive reaction with

erythromycin at the 20 min reading and four with only positive reactions (with amoxycillin or pseudoephedrine) at the 24 h reading. Among the three patients with initial clinical features of pruritus, one had a positive prick test reaction with tetrazepam at the 20 min reading. Among the patients with initial clinical features of urticaria, four of 13 (31%) had positive prick test reactions with amoxycillin, cefapirin, acetaminophen (paracetamol) or spiramycin at the 20 min reading. Among the four patients with initial features of angioedema, one had a positive prick test reaction with pholcodine at the 20 min reading (patient 64) with a slight relapse of angioedema and ocular pruritus. In urticaria and angioedema patients, prick tests were positive in five of 17 cases (29%). No false-positive prick test results were observed in any of the negative control subjects.

Intradermal tests

Among the 30 patients undergoing IDRs, 20 had positive results (67%), four on immediate readings only (two MPRs and two urticarias), 12 on delayed readings only (with amoxycillin, erythromycin, enoxoparin and piroxicam) and four on both immediate and delayed readings (with amoxycillin, piroxicam and oestradiol) (Tables 2–4). In one patient (no. 53), the IDR result with enoxoparin only became positive on day 3. IDR results were interpreted based on the reaction of negative control subjects, which allowed the detection of non-specific irritant reactions. Irritant reactions were observed with penicillin G, erythromycin and spiramycin. In our experience, test concentrations must be considered carefully, as too high a concentration may yield false-positive results. The highest concentration levels selected in this study were as follows: 5×10^5 U/mL for penicillin G, 0.2 mg/mL for erythromycin and 375 U/mL for spiramycin.

Altogether, IDR results were positive for eight of the 12 (67%) tested patients exhibiting MPR, four of the five patients with eczema and five of the 10 patients with urticaria and/or angioedema. After positive IDR reactions, one patient had localized MPR (patient 49), one had urticaria (patient 22) and one had slight angioedema (patient 38). As a whole, 52 of 72 patients (72%) manifested at least one positive, pertinent skin test result. No positive reactions on patch, prick or IDR testing with preservatives, colouring agents and excipients were observed in any patient.

Statistical analysis

In order to appreciate whether the type of ADR influenced

the rate of positive skin test reactions, we compared the results of these tests between the various clinical features of ADRs. A significantly higher ($P = 0.001$) number of positive patch test results was observed in MPRs (59%) when compared with urticarias (11%). We also compared the results of prick tests and IDRs performed in patients who had patch tests with negative results. In MPR patients, five of 12 (42%) had negative results of patch tests but positive prick tests or IDRs, while in urticaria patients, 11 of 13 (85%) had negative results of patch tests, but positive prick tests or IDRs ($P = 0.2$). In patients with patch tests with negative results, a higher number of positive prick test results was observed in urticaria patients (five of 17, 29%) when compared with MPR patients (0%) ($P = 0.32$).

Discussion

The results obtained in this prospective study involving 72 patients emphasize the value of drug skin tests with the suspected drug in defining the cause, and in monitoring, cutaneous ADRs, as positive results were obtained in 43%, 24% and 67% of (photo)patch, prick and IDR drug skin tests, respectively. In the case of prick tests and IDRs, they were performed in preselected patients with negative patch test results. Among 72 patients who had a very likely intrinsic imputability¹⁶ of one drug at the onset of their cutaneous ADR, 72% had a positive skin test reaction, using a combination of patch (plus photopatch tests in four patients¹⁸), prick and IDR tests. It should be emphasized that, in this series, 31 patients had had a readministration of the responsible drug with a relapse, often severe, of the cutaneous ADR before they were examined in our department. Nine patients had a relapse of the cutaneous ADR following the investigations.

It is necessary to define the value of the relatively safe drug skin tests in order to avoid, whenever possible, drug rechallenges. Some authors have considered re-exposure to the responsible drug for cutaneous ADR ethically inadvisable if severe cutaneous manifestations,¹⁹ especially EM or TEN,²⁰ occurred during the drug intake. Patch testing has proved to be a very safe method for the monitoring of cutaneous ADRs.^{5,9,15} Osawa *et al.*¹⁴ have demonstrated that positive patch test results were obtained in 31.5% of 197 patients manifesting cutaneous ADRs. Bruynzeel *et al.*¹¹ were able to obtain positive patch test results in 30–35% of patients suspected of being allergic to penicillin. In the data reported here, 43% of the patients had positive results with drug patch or photopatch tests.

These data emphasize that drug patch test results depend on: (i) clinical features and immunological mechanisms involved in cutaneous ADRs; (ii) the drug responsible for cutaneous ADRs and the manner in which tests are performed and read; and (iii) the specificity needing to be verified using appropriate negative control subjects.

Drug patch test results depend on the initial clinical features of the cutaneous ADR, especially if the cutaneous ADR is a T-cell-mediated immune drug reaction.^{3,6} In this study, 59% of patients with MPRs had positive patch test results; this was significantly different ($P = 0.001$) from the results observed in patients with urticarias (11%), therefore confirming the value of patch tests in monitoring MPRs.^{7,12} Bruynzeel and van Ketel⁷ have reported similar results in patients with MPRs caused by amoxycillin (18 of 47 positive patch test results). Our previous immunohistological studies¹² have supported the hypothesis of a delayed T-cell-mediated immune reaction in patients with MPRs induced by amoxycillin, accurately reproduced by drug skin tests with delayed positivity. Similar immunohistological alterations were found *in situ* in biopsy specimens obtained from MPRs induced by amoxycillin and in biopsy specimens obtained from the same patients who had delayed positive results of their patch tests, prick tests and/or IDRs performed with amoxycillin.¹² Patch tests may also be useful in determining the culprit drug in some other clinical features of cutaneous ADRs—thought to be induced by a cellular delayed immune reaction^{1–3}—such as AGEF,¹³ erythrodermas^{14,21,22} and generalized eczema.^{14,21} Photopatch tests have an investigative value in drug photosensitivity.^{18,23,24} Patch tests appear to be of less investigative value in determining the cause of EM, TEN¹³ or urticaria.¹⁵ Patch tests seem to be more sensitive if performed on the site of a previously affected area of skin, especially in FDEs.^{25,26} This may also hold true for other clinical types of cutaneous ADRs. Klein *et al.*²⁷ observed positive patch test reactions to co-trimoxazole only when patch tests were performed on the area previously affected by TEN.

Patch test results vary with the drug suspected as the cause of the cutaneous ADR. As confirmed by our data, patch tests seem to be useful in determining the culprit drug when they are performed with beta-lactam antibiotics, especially amoxycillin and cephalosporins,^{7–12,28} carbamazepine,^{22,29–31} tetrazepam,³² pristinamycin,²¹ heparin,^{33,34} pseudoephedrine,³⁵ corticosteroids,^{36,37} diltiazem³⁸ and hydroxyzine.³⁹ They may also be of diagnostic value for other drugs,⁹

such as diclofenac,⁴⁰ diazepam,¹⁵ meprobamate¹⁵ and co-trimoxazole.²⁷

False-negative drug patch test results can be attributable to various causes. The drug being tested may be unable to penetrate into the epidermis, or the concentration used in patch testing may be too low to elicit a positive reaction. To avoid this, tests were carried out with pure drugs and diluted only by 30%, the highest concentration giving a good distribution of the drug into the white petrolatum. The vehicle with which the drug was diluted may not enhance the penetration of the tested drug into and through the epidermis. For this reason, we patch tested each drug with different vehicles²⁵ (e.g. in patient 13, a positive patch test reaction was obtained only when amoxycillin was diluted in petrolatum). Another explanation, of course, is that the culprit source of the cutaneous ADR was not the native drug itself but one of its metabolites.¹⁴ Such a mechanism was noted recently by Popescu *et al.*⁴¹ who demonstrated using prick tests and IDR that five patients with allergic reactions to cyclophosphamide had immediate positive skin tests when performed with two cyclophosphamide metabolites but not with the native drug. Appropriate delayed readings have to be performed. In our study, immediate and delayed readings were performed at 20 min, on days 2 and 4 and, whenever possible, on day 7. Four patients (nos 6, 29, 31 and 50) with negative patch test reactions on days 2 and 3 had positive readings on day 4.

The diagnostic value of prick tests and IDRs in determining the causes of cutaneous ADRs has been less evaluated than the value of patch tests. Osawa *et al.*¹⁴ obtained positive IDR results in 90% of their patients but, in the absence of appropriate negative controls, the specificity of their IDRs cannot be validated. Most papers published on drug prick tests and IDRs relate to ADRs induced by beta-lactam antibiotics.^{10,12,42–44} Prick tests and IDRs have been performed less frequently with other drugs such as diltiazem,³⁸ heparin,^{33,34} insulin,⁴⁵ oestradiol⁴⁶ or corticosteroids.⁴⁷ In this study, using an unusually large pool of patients, prick tests and IDRs were performed with 23 and 12 different drugs, respectively.

When patch test results are negative, prick tests and IDRs can be performed and can be of diagnostic value. In this study, 21 patients who had negative results for their drug patch tests had positive results for drug prick tests or IDRs. In urticaria patients, when patch test results are negative, prick tests seem to have a higher diagnostic value than in MPRs. As reported previously, we observed delayed positive IDR results in cutaneous

ADRs caused by amoxycillin^{10,12,48} or heparin.^{33,34} We also observed delayed positive prick tests results with pseudoephedrine and delayed positive IDR results with piroxicam and erythromycin. Caution must be used when performing drug skin tests. Even if patch tests seem to be the safest, we emphasize that they are also able to reinduce an ADR. This was observed in this study when using aciclovir, amoxycillin, hydroxyzine, pseudoephedrine or triamcinolone. This phenomenon has also been reported with pseudoephedrine,³⁵ carbamazepine²⁹ and clobazepam.⁴⁹

When patients had an initial clinical presentation of urticaria or angioedema, prick tests were performed with diluted preparations of the suspected drug. Even with this precaution, one patient (no.64) had a relapse of angioedema. The risk of relapse of initial cutaneous ADR is even greater when performing IDRs. Among 30 IDRs performed, three incidents were observed (one MPR, one urticaria and one angioedema). Because of this risk, we suggest that the guidelines described in the Patients and methods section be strictly adhered to. The volume of drug administered for IDRs should be as low as possible. In our experience, 0.04 mL is the safest quantity and the lowest volume that gives reproducible responses.

Calkin and Maibach⁹ stressed the need for at least 20 negative control subjects in order to report drug patch test results. This also appears to be necessary for reporting prick and IDR drug test results. In this study, it was ascertained that all negative control patients had been treated with the drug in question within 6 weeks of being tested, as had the allergic patients. Such negative control subjects are difficult to collect, especially for drugs not widely used or well tolerated, but they seem to be better than healthy subjects, first from an ethical point of view and, secondly, because, regarding drug intake, negative control patients have the same history of use of the drug as the patients studied. Positive patch test results seem specific compared with negative controls, as only one doubtful false-positive patch test result was observed among seven negative control subjects tested with captopril. Negative control subjects for interpreting IDR results are absolutely necessary to avoid the declaration of a false-positive result, as we determined for penicillin, erythromycin and spiramycin. The high percentage of positive test results (72%) observed in this study is due to the careful following of the guidelines proposed herein. It must be emphasized that: (i) negative results in skin tests do not exclude the responsibility of a drug for the onset of a cutaneous ADR, and (ii) it is practicable to

compare test results with specifically selected negative patient controls. Logically, the first tests to be performed should be patch tests (confirmed by readings taken after at least 96 h). Prick tests can then be performed (obviously using sequential increasing dilutions in patients with urticaria or angioedema), especially to investigate urticarias. Only when both patch and prick tests are negative should IDRs be performed, with sterilized sequential dilutions in hospitalized patients. Both prick tests and IDR readings should be made at 20 min, 6 h and 24 h at least and, whenever possible, confirmed on day 3. Excipients, preservatives and colouring agents have to be tested as well, even if they are an infrequent cause of cutaneous ADRs and preferentially induce urticaria or anaphylactic reactions.⁵⁰

In conclusion, this study demonstrates the feasibility and value of careful sequential drug testing for the determination of the cause of cutaneous ADRs and proposes guidelines for performing such investigations. In this type of sequential drug testing, it is imperative to remember that all testing should be halted as soon as a confirmed positive drug test has been obtained. This must be done in order to avoid a relapse of the initial cutaneous ADR, which, among other things, can be caused by clinical intolerance to these tests.

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References

- 1 Breathnach SM. Mechanisms of drug eruptions. Part 1. *Australas J Dermatol* 1995; **36**: 121–7.
- 2 de Weck AL. Pharmacologic and immunochemical mechanisms of drug hypersensitivity. *Immunol Allergol Clin N Am* 1991; **11**: 461–74.
- 3 Barbaud A, Béné M-C, Faure G. Immunological physiopathology of cutaneous adverse drug reactions. *Eur J Dermatol* 1997; **7**: 319–23.
- 4 Ressler C, Mendelson LM. Skin test for diagnosis of penicillin allergy. Current Status. *Ann Allergy* 1987; **59**: 167–70.
- 5 Bruynzeel DP, van Ketel WG. Patch testing in drug eruptions. *Semin Dermatol* 1989; **8**: 196–203.
- 6 Breathnach SM. Management of drug eruptions. Part II. Diagnosis and treatment. *Australas J Dermatol* 1995; **36**: 187–91.
- 7 Bruynzeel DP, van Ketel WG. Skin tests in the diagnosis of maculopapular drug eruptions. *Semin Dermatol* 1987; **6**: 119–24.
- 8 Romano A, Di Fonso M, Pietrantonio F *et al*. Repeated patch testing in delayed hypersensitivity to beta-lactam antibiotics. *Contact Dermatitis* 1993; **28**: 190.
- 9 Calkin JM, Maibach H. Delayed hypersensitivity drug reactions diagnosed by patch testing. *Contact Dermatitis* 1993; **29**: 223–33.

- 10 Romano A, Di Fonso M, Papa G *et al.* Evaluation of adverse cutaneous reactions to aminopenicillins with emphasis on those manifested by maculopapular rashes. *Allergy* 1995; **50**: 113–18.
- 11 Bruynzeel DP, von Blomberg-van der Flier M, Scheper RJ *et al.* Penicillin allergy and the relevance of epicutaneous tests. *Dermatologica* 1985; **171**: 429–34.
- 12 Barbaud A, Béné M-C, Schmutz J-L *et al.* Role of delayed cellular hypersensitivity and adhesion molecules in amoxycillin-induced morbilliform rashes. *Arch Dermatol* 1997; **133**: 481–6.
- 13 Wolkenstein P, Chosidow O, Fléchet M-L *et al.* Patch testing in severe cutaneous adverse drug reactions, including Stevens–Johnson syndrome and toxic epidermal necrolysis. *Contact Dermatitis* 1996; **35**: 234–6.
- 14 Osawa J, Naito S, Aihara M *et al.* Evaluation of skin test reactions in patients with non-immediate type drug eruptions. *J Dermatol* 1990; **17**: 235–9.
- 15 Felix R, Comaish J. The value of the patch tests and other skin tests in drug eruptions. *Lancet* 1974; **i**: 1017–19.
- 16 Moore N, Paux G, Begaud B *et al.* Adverse drug reaction monitoring: doing it the French way. *Lancet* 1985; **ii**: 1056–8.
- 17 Adams RM, Fischer T. Diagnostic patch testing. In: *Occupational Skin Diseases* (Adams RM, ed.), 2nd edn. Philadelphia: WB Saunders Co., 1990; 223–53.
- 18 Jeanmougin M. Détermination du pouvoir photosensibilisant d'un médicament par la méthode des photopatch tests. *Nouv Dermatol* 1986; **5**: 204–8.
- 19 Sacerdoti G, Vozza A, Ruocco V. Identifying skin reactions to drugs. *Int J Dermatol* 1993; **32**: 469–79.
- 20 Kauppinen K, Alanko K. Oral provocation: uses. *Semin Dermatol* 1989; **8**: 187–91.
- 21 Michel M, Dompmmartin A, Szczurko C *et al.* Eczematous-like drug eruption induced by synergists. *Contact Dermatitis* 1996; **34**: 86–7.
- 22 Jones M, Fernandez-Herrera J, Dorado JM *et al.* Epicutaneous test in carbamazepine cutaneous reactions. *Dermatology* 1994; **188**: 18–20.
- 23 Hölzle E, Neumann N, Hausen B *et al.* Photopatch testing: the 5-year experience of the German, Austrian, and Swiss photopatch test group. *J Am Acad Dermatol* 1991; **25**: 59–68.
- 24 Menz J, Muller SA, Connolly S. Photopatch testing: a six-year experience. *J Am Acad Dermatol* 1988; **18**: 1044–7.
- 25 Alanko K, Stubb S, Reitamo S. Topical provocation of fixed drug eruption. *Br J Dermatol* 1987; **116**: 561–7.
- 26 Sehgal VN, Gangwani OP. Fixed drug eruption. Current concepts. *Int J Dermatol* 1987; **26**: 67–74.
- 27 Klein CE, Trautmann A, Zillikens D, Bocker EB. Patch testing in an unusual case of toxic epidermal necrolysis. *Contact Dermatitis* 1995; **33**: 448–9.
- 28 Adkinson NE. Side-chain specific beta-lactam allergy. *Clin Exp Allergy* 1990; **20**: 445–7.
- 29 Vaillant L, Camenen I, Lorette G. Patch testing with carbamazepine: reinduction of an exfoliative dermatitis. *Arch Dermatol* 1989; **125**: 299.
- 30 Alanko K. Patch testing in cutaneous reactions caused by carbamazepine. *Contact Dermatitis* 1993; **29**: 254–7.
- 31 Puig L, Nadal C, Fernandez-Figueras M-T, Alomar A. Carbamazepine-induced drug rashes: diagnostic value of patch tests depends on clinico-pathologic presentation. *Contact Dermatitis* 1996; **34**: 435–7.
- 32 Ortega NR, Barranco P, Lopez Serrano C *et al.* Delayed cell-mediated hypersensitivity to tetrazepam. *Contact Dermatitis* 1996; **34**: 139.
- 33 Bircher AJ, Flückiger R, Buchner SA. Eczematous infiltrated plaques to subcutaneous heparin: a type IV allergic reaction. *Br J Dermatol* 1990; **123**: 507–14.
- 34 Moreau A, Dompmmartin A, Esnault P *et al.* Delayed hypersensitivity at the injection sites of a low-molecular-weight heparin. *Contact Dermatitis* 1996; **34**: 31–4.
- 35 Tombs RR, Lepoittevin JP, Espinassouze F *et al.* Systemic contact dermatitis from pseudoephedrine. *Contact Dermatitis* 1991; **24**: 86–8.
- 36 Doooms-Goossens A. Allergy to inhaled corticosteroids: a review. *Am J Contact Dermatitis* 1995; **6**: 1–3.
- 37 Bircher AJ, Pelloni F, Langauer-Messmer S, Müller D. Delayed hypersensitivity reactions to corticosteroids applied to mucous membranes. *Br J Dermatol* 1996; **135**: 310–13.
- 38 Romano A, Pietrantonio F, Garcovich A *et al.* Delayed hypersensitivity to diltiazem in two patients. *Ann Allergy* 1992; **69**: 31–2.
- 39 Michel M, Dompmmartin A, Louvet S *et al.* Skin reactions to hydroxyzine. *Contact Dermatitis* 1997; **36**: 147–9.
- 40 Romano A, Pietrantonio F, Di Fonso M *et al.* Positivity of patch tests in cutaneous reaction to diclofenac. *Allergy* 1994; **49**: 57–9.
- 41 Popescu NA, Sheehan MG, Kouides PA *et al.* Allergic reactions to cyclophosphamide: delayed clinical expression associated with positive immediate skin tests to drug metabolites in five patients. *J Allergy Clin Immunol* 1996; **97**: 26–33.
- 42 Blanca M, Vega JM, Garcia J *et al.* Allergy to penicillin with good tolerance to other penicillins: study of the incidence in subjects allergic to betalactams. *Clin Exp Allergy* 1990; **20**: 475–81.
- 43 Silviu-Dan F, McPhillips S, Warrington R. The frequency of skin test reactions to side-chain penicillin determinants. *J Allergy Clin Immunol* 1993; **91**: 694–701.
- 44 Audicana M, Bernaola G, Urrutia I *et al.* Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. *Allergy* 1994; **49**: 108–13.
- 45 Barbaud A, Got I, Tréchet P *et al.* Allergies cutanées et insulinothérapie. Aspects récents, conduite à tenir. *Ann Dermatol Venerol* 1996; **123**: 214–18.
- 46 Shelley WB, Shelley ED, Talanin NY, Santoso-Pham J. Estrogen dermatitis. *J Am Acad Dermatol* 1995; **32**: 25–31.
- 47 Vidal C, Tome S, Fernandez-Redondo V, Tato F. Systemic allergic reaction to corticosteroids. *Contact Dermatitis* 1994; **31**: 273–4.
- 48 Warrington RJ, Silviu-Dan F, Magro C. Accelerated cell-mediated immune reactions in penicillin allergy. *J Allergy Clin Immunol* 1993; **92**: 626–8.
- 49 Machet L, Vaillant L, Dardaine VF, Lorette G. Patch testing with clobazepam: relapse of generalized drug eruption. *Contact Dermatitis* 1992; **26**: 347–8.
- 50 Barbaud A. Place of excipients in drug related allergy. *Clin Rev Allergol Immunol* 1995; **13**: 253–63.