
Delayed hypersensitivity to cefazolin: report on a case involving lymphocyte transformation studies with different cephalosporins

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Background: A cell-mediated immunopathogenic mechanism has been demonstrated in only a few cases of cutaneous reactions to systemically administered cephalosporins.

Objective: The aim was to investigate the pathogenic mechanism of a maculopapular rash experienced by a subject during cefazolin therapy.

Methods and Results: Prick, intradermal, and patch tests were carried out using penicillin determinants, ampicillin, amoxicillin, cefazolin, cephalothin, cefuroxime, ceftazidime, and ceftriaxone. Those tests for penicillin G and its determinants, as well as for ampicillin and amoxicillin, were negative. The patient displayed patch-test and delayed intradermal-test positivity to all the cephalosporins tested. No specific immunoglobulin E antibodies were found for penicillins or cefazolin. The lymphocyte-transformation-test results were negative for all the penicillins tested and showed a positive concentration-effect curve for cefazolin, ceftazidime, and ceftriaxone at concentrations up to 50 $\mu\text{g/mL}$. At 100 $\mu\text{g/mL}$ the responses decreased with all the cephalosporins tested. Challenges with penicillin G and amoxicillin were well tolerated, but the challenge with cefazolin was positive.

Conclusions: The data of this case demonstrate delayed hypersensitivity to cefazolin. Patch tests and delayed-reading intradermal tests can be a simple and effective means of diagnosing this type of reaction. Both in vivo and in vitro studies indicate that the responses were directed toward a determinant shared by all cephalosporins, but not by penicillins. The concentration of the cephalosporins used for the in vitro lymphocyte stimulation was critical, because at the concentrations normally used to test other β -lactams the response decreased. This phenomenon may be attributable to an immunosuppressive, rather than toxic, effect.

Ann Allergy Asthma Immunol 2001;87:238–242.

INTRODUCTION

Cefazolin, a first-generation cephalosporin, is a β -lactam antibiotic widely used for surgical prophylaxis because of its effectiveness in nosocomial infections by Gram-negative organisms. Cases of adverse reactions to this drug mediated by immune mechanisms have been described.^{1–5} Most of these

patients present a specific IgE-dependent reactivity to cefazolin, with good tolerance of other β -lactams, including other cephalosporins.^{2–4}

The frequency of cutaneous reactions in patients treated with cephalosporins ranges from 1% (cefuroxime) to 3% (ceftriaxone),⁶ but, to our knowledge, few studies have reported delayed hypersensitivity reactions to systemically administered cephalosporins,^{1,7–9} whereas there are many reports of such reactions to penicillins.^{9–20} On the basis of patch-test or delayed intradermal-test positivity, only one case of maculopapular reaction to cefazolin and one of occupational airborne contact dermatitis from

this drug seem to involve a cell-mediated pathogenic mechanism.^{1,5}

We present a case of delayed hypersensitivity to cefazolin. Our in vivo and in vitro studies show that a T-cell response was involved, as well as cross-reactivity with other cephalosporins. Good tolerance of penicillins was also demonstrated.

CASE REPORT

A 44-year-old woman was treated intramuscularly with cefazolin (1 g twice a day) as preoperative prophylaxis. During the second day of therapy, 6 hours after the administration of the third dose of the drug, she developed a generalized pruritic maculopapular rash. Treatment was immediately interrupted, and the rash resolved after 7 days of corticosteroid therapy (6-methylprednisolone intravenously, 40 mg daily). After this episode, the patient tolerated macrolides and sulfonamides on several occasions. She had no history of allergic diseases, whereas a brother had bronchial asthma and a son had allergic rhinitis. Nine years after the reactive episode, the patient came to our clinic to be evaluated for a possible allergy to β -lactams and, after obtaining her informed consent, the following allergy study was undertaken.

Skin Tests

Prick and intradermal tests were carried out using penicilloylpolyllysine (PPL; Allergopharma Merck, Reinbek, Germany), minor determinant mixture (MDM; Allergopharma), penicillin G (PG; Pharmacia, Milan, Italy), ampicillin (AM; Amplital; Pharmacia) and amoxicillin (AX; Velamox, SmithKline Beecham, Milan). The final concentration was 5×10^{-5} mmol/L for PPL, $2 \times$

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Received for publication April 25, 2000.

Accepted for publication in revised form November 10, 2000.

10⁻² mmol/L for MDM, 10,000 IU/mL for PG, 20 mg/mL for AM and AX (the last three were diluted in 0.9% NaCl).

Cefazolin (Cefamezin; Pharmacia), cefuroxime (Curoxim; Glaxo, Verona, Italy), cephalothin (Keflin; Lilly, Sesto Fiorentino, Italy), ceftazidime (Glazidim; Glaxo), and ceftriaxone (Rocefin; Roche, Basel, Switzerland) at a concentration of 2 mg/mL 0.9% NaCl were also used.

All the above reagents were initially tested on volar forearm skin by the prick method and reactions were considered positive when a wheal >3 mm in diameter was present 20 minutes later. When prick tests were negative, 0.01 mL of the reagent solution was injected intradermally on volar forearm skin. Readings were made 20 minutes and 24, 48, and 72 hours after injection. Results were considered positive when wheals of >5 mm were present. Positive controls for prick and intradermal tests were done with histamine (at 10 and 1 mg/mL, respectively). Normal saline was used as a negative control. The concentration used for cephalosporins proved to be nonirritant in a control group of 20 healthy subjects, 5 of whom had previously tolerated cefazolin.

Patch Tests

Patch tests were done with PG (5% in petroleum jelly), AM (5% in petroleum jelly), AX (5% in petroleum jelly) and the cephalosporins reported above (200 mg/mL in 0.9% NaCl). All reagents were applied to uninvolved skin on the interscapular region of the

patient's back, using acrylate adhesive strips with small plates attached for the test allergens (Curatest; Lohmann GmbH, Neuwied, Germany). Occlusion time was 48 hours. Readings were made as recommended by Wilkinson et al²¹ 15 minutes after removal of the strips and 24 hours later. Patch tests performed with all the reagents produced negative results in the control group.

Challenges

Challenges with PG (1,000,000 IU), cefazolin (1 g), and AX (1 g) were also performed, the first two intramuscularly and the last orally. Increasing doses (0.01, 0.1, and 1.0 of the respective therapeutic doses indicated in parentheses) were administered at 1-week intervals.

In Vitro Tests

RAST was performed for PG, AX, AM, and cefazolin as previously described.²² The lymphocyte transformation test (LTT) was carried out according to Nyfeler and Pichler.²³ PG, AX, and the cephalosporins were added to the medium at concentrations ranging from 2 to 500 µg/mL. Tetanus toxoid (Instituto Berna, Barcelona, Spain) was used as a control antigen at a concentration of 10 limit flocculation/mL. Stimulation indexes (SI) were calculated as counts per minute of antigen-containing cultures divided by counts per minute of control cultures (cultures without antigen). A SI > 2.0 was interpreted as a positive result. The LTT was also carried out in 10 subjects of the control group, of whom 2 had previously been treated

with cefazolin and 1 with cefuroxime without any adverse reaction.

RESULTS

Skin tests for penicillin determinants (PPL, MDM, and PG), AM, and AX were negative at the immediate (20 minutes) and delayed (24, 48, and 72 hours) readings. Intradermal skin tests for cephalosporins did not produce immediate reactions, but for all of them, the 24-hour reading showed erythematous, indurated wheals larger than 10 mm, which reached their maximum size at 48 hours and began to fade at 72 hours.

Patch tests for PG, AM, and AX were negative at the 48-hour and 72-hour readings. Cefazolin, cefuroxime, cephalothin, and ceftazidime produced positive reactions (2+), consisting of indurated, erythematous areas approximately 18 mm in diameter with vesicles. Ceftriaxone produced an indurated, erythematous lesion 13 mm in diameter (1+). The positivities were present at the 48- and 72-hour readings.

No specific IgE antibodies were found for any determinants. The concentration of the optimal response in the LTT for each hapten is shown in Figure 1. Results were negative for PG, AX, and cefuroxime at all the concentrations used, but, at 50 µg/mL, they were positive for ceftazidime, cefazolin, and ceftriaxone, and borderline for cephalothin. LTT responses to four different concentrations of cephalosporins in the patient are presented in Figure 2 and the mean values in Figure 3. Central tendency and percentile values in the control group are also shown in Table 1. As can be seen, the SI decreases at the highest concentration shown. In the patient, the 100-µg/mL concentration produces lower responses with ceftazidime, ceftriaxone, and cefazolin, but the SI still remains > 2 (Fig 2). In the control group, the SI also decreases at the highest concentration (Fig 3). None of them had SI values > 2 for any of the drugs or concentrations tested. SI values in the patient were always higher than the 90th percentile values in the control group, with the exceptions of

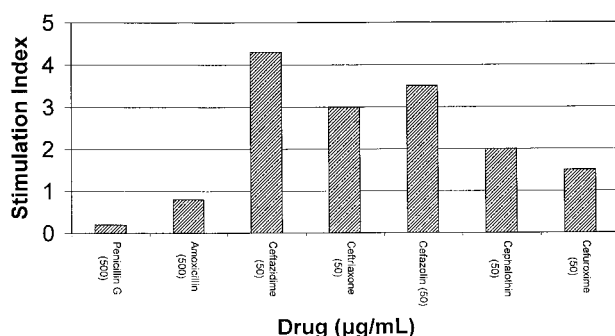


Figure 1. The patient's LTT results for each drug at the concentration that produced the highest stimulation index.

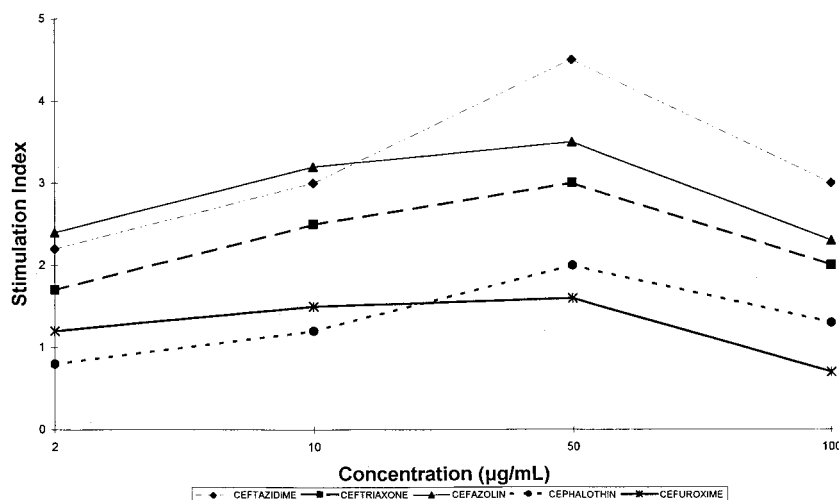


Figure 2. The patient's LTT responses to different concentrations of cephalosporins.

cefuroxime (2, 10, and 50 µg/mL) and cephalothin (2 µg/mL).

Challenges with PG and AX were well tolerated. The patient reacted to the 100-mg dose of cefazolin, developing an exanthematic eruption on her abdomen and legs 12 hours after the administration of the drug.

DISCUSSION

A number of studies have shown that maculopapular rashes occurring during β -lactam therapy often represent type-IV (cell-mediated) hypersensitivity, which is associated with patch-test and/or delayed (ie, > 6 hours after administration) intradermal-test positivity.^{1,7-20} In some studies,^{1,8,9,11,13,14,18,20} challenges with the suspect drugs have induced delayed-onset skin rashes, confirming the diagnosis. Our patient had suffered a maculopapular rash on the second day of cefazolin therapy. In vivo and in vitro studies, including the challenge, showed that this was a cell-mediated reaction. The absence of skin-test and patch-test positivity for the penicillins tested, as well as negative results in the LTT performed with these drugs, was fully confirmed by the patient's tolerance of challenges with PG and AX. This indicates that the response was directed toward a determinant shared by all cephalosporins (probably the six-membered dihydrothiazide-ring), but not by any penicillin. This lack of cross-reactivity between

penicillins and cephalosporins at the T-cell level has been previously described.²⁴ Although the possibility of co-existent sensitivity to cephalosporins other than cefazolin can not be ruled out, it seems highly unlikely because the patient had never been administered any such drugs.

Our experience regarding delayed reactions to aminopenicillins suggests that patch-test positivity together with delayed intradermal-test positivity is an indicator of delayed hypersensitivity. In our previous study, all 16 patients who displayed this pattern of

positivity and agreed to undergo oral challenges with the culprit aminopenicillins reacted positively.¹³ Since we did not have sufficient experience with nonimmediate cutaneous reactions to cephalosporins, we nevertheless challenged this one patient. We now believe, however, that in patients who have suffered maculopapular reactions to β -lactams, including cephalosporins, the above pattern of skin-test positivity is sufficient for the diagnosis.

The concentration of the cephalosporins used for the in vitro lymphocyte stimulation was critical, because at a concentration of 100 µg/mL, which is lower than those normally used to test other β -lactams,²³ the response decreased in the patient as well as in the control group. Similar results with regard to healthy adults have been obtained in LTTs with cephalosporins such as cephalothin and cefoxitin by Rouveix et al.²⁵ Their data suggest that this phenomenon may be attributable to a significant immunomodulating activity on human lymphocyte subsets, possibly by activating suppressor cells. At the highest concentrations, however, a toxic effect cannot be excluded. The immunosuppressive effect, produced by most cephalosporins, could contribute to the lower incidence of delayed hypersensitivity reactions observed with these drugs compared with

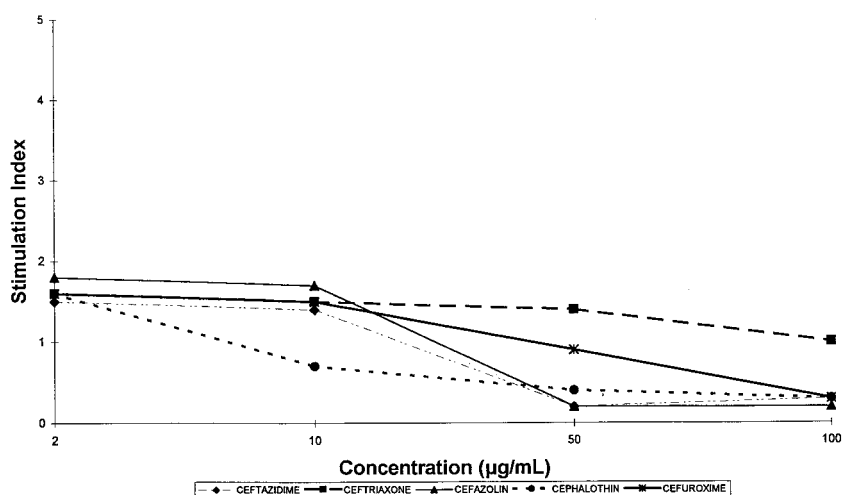


Figure 3. Mean values of the control group's LTT responses to different concentrations of cephalosporins.

Table 1. Frequencies of the SI in the Control Group: Central Tendency and Percentile (P) Values

Drug ($\mu\text{g/mL}$)	Mean	Median	SD	P ₂₅	P ₇₅	P ₉₀	Patient's SI values
Ceftazidime (2)	1.50	1.57	0.22	1.47	1.62	1.66	2.23
Ceftazidime (10)	1.41	1.43	0.32	1.16	1.68	1.95	3.01
Ceftazidime (50)	0.20	0.16	0.14	0.10	0.30	0.43	4.49
Ceftazidime (100)	0.31	0.24	0.20	0.17	0.50	0.68	3.01
Ceftriaxone (2)	1.60	1.62	0.07	1.51	1.66	1.69	1.71
Ceftriaxone (10)	1.50	1.43	0.22	1.35	1.67	1.85	2.50
Ceftriaxone (50)	1.40	1.40	0.32	1.03	1.65	1.90	3.14
Ceftriaxone (100)	1.00	0.90	0.53	0.65	1.23	1.98	2.06
Cefazolin (2)	1.80	1.87	0.20	1.55	1.96	1.99	2.41
Cefazolin (10)	1.70	1.70	0.17	1.64	1.83	1.93	3.22
Cefazolin (50)	0.24	0.24	0.16	0.08	0.41	0.43	3.53
Cefazolin (100)	0.20	0.22	0.11	0.07	0.27	0.39	2.28
Cephalothin (2)	1.59	1.54	0.25	1.41	1.83	1.95	0.81
Cephalothin (10)	0.71	0.69	0.18	0.58	0.90	0.99	1.24
Cephalothin (50)	0.40	0.34	0.25	0.17	0.62	0.83	2.01
Cephalothin (100)	0.30	0.22	0.23	0.13	0.50	0.74	1.35
Cefuroxime (2)	1.62	1.66	0.31	1.35	1.90	1.99	1.19
Cefuroxime (10)	1.49	1.41	0.27	1.29	1.69	1.96	1.51
Cefuroxime (50)	0.90	0.76	0.44	0.61	1.07	1.86	1.62
Cefuroxime (100)	0.29	0.24	0.19	0.14	0.48	0.61	0.75

Bold numbers correspond to results over P₉₀

penicillins, although this hypothesis has not yet been evaluated. The negative results of the LTT for cephalothin and cefuroxime, despite positive patch and intradermal tests, may be explained by the possibly greater sensitivity of the latter tests.

An interesting observation is that the patient had suffered her reaction 9 years before we began the study and has not taken the drug since then. This confirms our findings with aminopenicillins that delayed hypersensitivity is a long-lasting condition,^{13,20,26} unlike the immediate type, which tends to disappear with the passing of time if the culprit drug is avoided. A recent study on subjects allergic to penicillins shows that negativization of skin tests can be observed as early as 1 year after performance of the first allergologic evaluation,²⁷ whereas subjects with delayed allergic reactions can remain positive for 6 years or longer after the reactive episode.^{13,20}

CONCLUSION

This case of delayed hypersensitivity to cefazolin, shows that patch tests and delayed-reading intradermal tests can

be a simple and effective tool for diagnosing this type of reaction. Both in vivo and in vitro studies confirm that a T-cell response was involved and that it was directed toward a determinant shared by all cephalosporins, but not by penicillins.

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