

Incidence of β -Lactam–Induced Delayed Hypersensitivity and Neutropenia During Treatment of Infective Endocarditis

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Background: Long-term parenteral β -lactam treatment is often complicated by adverse reactions that necessitate drug withdrawal.

Objective: To evaluate the incidence and mechanism of β -lactam adverse reactions during an 8-year period in all episodes of suspected infective endocarditis in patients treated at a university-affiliated institution.

Methods: Patients with 215 consecutive episodes of β -lactam treatment for 10 days or more were prospectively enrolled during 2 periods, January 1984 through December 1988 and January 1993 through December 1995, and compared with 51 episodes of vancomycin hydrochloride treatment for 10 days or more. Incidents of adverse reactions, such as fever, rash, or neutropenia, were registered. Neutrophil counts, eosinophil counts, and penicillin antibodies were studied. Patients with delayed adverse reactions to penicillin G sodium were re-challenged with penicillin v potassium.

Results: Incidence of delayed adverse reactions during treatment was 33% with β -lactams compared with 4% with vancomycin. Rates of adverse event for β -lactams increased continuously from treatment day 15 to day 30. A 6-fold difference in capacity to induce adverse events

was found with different β -lactams. Penicillin G induced neutropenia in 14% and any adverse event in 51% of treated episodes. Mean daily doses significantly influenced the frequency of adverse events. Occurrence of hemagglutinating penicillin antibodies was significantly related to patients whose penicillin-treated episodes were complicated with adverse events. Patients with delayed adverse reactions to penicillin G were safely re-challenged with penicillin.

Conclusions: Incidence of delayed adverse reactions to β -lactams increases sharply when parenteral treatment is extended beyond 2 weeks. Penicillin G is the most frequent inducer of adverse reactions among β -lactams studied. An immunological reaction mediated by antibodies to the penicilloyl determinant may be involved in the pathogenesis, possibly enhanced by a dose-related toxic trigger mechanism. β -Lactam–induced neutropenia followed a uniform pattern, occurring after, on average, 21 days of treatment, and might be due to both immunologic and toxic effects of treatment. Patients with a late adverse reaction to penicillin can safely be re-treated with penicillin, although they should remain under close surveillance if treatment extends beyond 2 weeks.

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USE OF β -LACTAM antibiotics (penicillins and cephalosporins), given in large doses intravenously, is a cornerstone of treating many severe bacterial infections. Large doses of β -lactam antibiotics for periods as long as 2 to 6 weeks are often used to ensure eradication of bacteria from the heart valves in infective endocarditis (IE). β -Lactams are generally considered remarkably nontoxic within a broad dose range. However, they induce a variety of adverse reactions, most of which are believed to be immune mediated.

The frequency of adverse events during long-term parenteral β -lactam treatment is high and causes clinical problems.

The mechanism of these adverse events is not known in detail, which often forces the clinician to establish a diagnosis of β -lactam allergy. Patients with a history of an adverse reaction to a β -lactam antibiotic are often denied new treatment with this antibiotic because of fear of an immediate type of allergic reaction. In clinical practice, however, several of these patients have received new β -lactam treatment courses without any complications.

Severe neutropenia with onset during the third or fourth treatment week has been reported in 5% to 15% of treated episodes.¹⁻⁹ In another study,¹⁰ when patients were treated with a mean daily dose (MDD) of 16.7 g/d of penicillin G sodium, we found an incidence of neutropenia of up to 50%.

PATIENTS AND METHODS

A prospective consecutive study of 229 episodes of suspected IE in 205 patients treated at the Department of Infectious Diseases, Sahlgrenska University Hospital/Östra, Göteborg, Sweden, was performed during 8 years: January 1984 through December 1988 and January 1993 through December 1995. A standardized regimen for antibiotic treatment, surgical interventions, weekly blood sampling, and follow-up was used, with only minor changes during the period studied.

A combination of an aminoglycoside and a β -lactam antibiotic was given for a median of 2 weeks, followed by an intravenous β -lactam antibiotic for a total treatment period of 4 weeks in uncomplicated episodes. All 215 courses of treatment with a β -lactam antibiotic of 10 days or more (penicillin G, cloxacillin sodium, ampicillin sodium, piperacillin sodium, or cefuroxime sodium) are included in the study. During the first 2 years of the study, initial doses of 18 g/d of penicillin G sodium were given; these doses were lowered to 12 g/d during the remaining period. The initial dose of cloxacillin sodium, ampicillin sodium, and piperacillin sodium was 12 g/d and of cefuroxime sodium was 6.75 g/d throughout the study. All β -lactam antibiotic solutions were freshly prepared (≤ 1 hour) and given as intravenous bolus injections every 8 hours with the exception of cloxacillin, which was administered as an intravenous infusion every 8 hours for 15 to 30 minutes. Vancomycin hydrochloride was given twice daily as an intravenous infusion for 30 to 60 minutes.

Detailed analyses of epidemiology, early surgical interventions, diagnostic criteria, and inflammatory variables during treatment have been published elsewhere.¹⁶⁻¹⁹

An adverse event was defined as a clinical or laboratory event causing discontinuation of antibiotic therapy,

and included fever, maculopapular rashes, neutropenia, diarrhea, thrombophlebitis, and serum sickness syndrome. Criteria for assessing the causal relation between a given drug therapy and neutropenia, rash, or fever were defined according to Kramer et al.²⁰ Adverse reactions such as neutropenia, rash, or fever were thus considered to be induced by a drug only if they followed a reasonable time sequence from administration of the drug, disappeared on stopping administration of the drug, and were not explained by the patient's clinical condition.

Fever was defined as a rectal temperature of 38°C or higher measured on at least 2 occasions and was considered to be drug related if the definitions according to Kramer et al²⁰ were fulfilled and a minimum of 2 afebrile days had elapsed after the initial fever had disappeared.

Peripheral white blood cell counts, neutrophil counts, and eosinophil counts were determined using routine methods. Neutropenia was defined as a neutrophil count in peripheral blood of $1.0 \times 10^9/L$ or less. Eosinophilia was defined as an absolute count in peripheral blood of $0.750 \times 10^9/L$ or more.

ANALYSIS OF CLINICAL DATA

The total dose of an antibiotic given for each episode with and without adverse reactions was determined and the MDD was then calculated. All episodes with antibiotic treatment courses shorter than 10 days were excluded. If a patient was treated sequentially with more than 1 antibiotic for 10 days or more, every antibiotic treatment course was regarded as a separate episode. If more than 1 antibiotic drug was given and an adverse reaction occurred, the drug given while the adverse reaction developed was considered the main cause. In only 1 episode were 2 β -lactams given simultaneously when an adverse reaction developed, and they were both considered to be causative agents.

A clinical syndrome of neutropenia, numerous neutrophil precursors, lack of well-differentiated myeloid elements in bone marrow aspirates, and lack of complete recovery 1 to 7 days after discontinuation of β -lactam therapy has been reported in several series.^{1,11-15} It is not known whether β -lactam-induced neutropenia is a result of direct toxic effects to the bone marrow or whether it is immune mediated.

We conducted a prospective study on all patients with IE admitted to our hospital during an 8-year period, with special emphasis on delayed adverse reactions to β -lactam antibiotics in relation to different dosage regimens. Studies of penicillin antibodies and results from challenges with penicillin in patients with late adverse reactions to penicillin are reported.

RESULTS

DEMOGRAPHICS AND OUTCOME

Of 229 consecutive suspected IE episodes treated with a full treatment course during the 8-year period studied, 193 episodes (84.3%) could be diagnosed as IE using modified von Reyn criteria.^{18,25} The newly proposed DUKE criteria²⁶ could categorize 167 episodes (72.9%) as defi-

nite. The median age was 64 years (range, 19-90 years), and the male-female ratio was 130:99. A predisposing cardiac disease was identified in 65% of the episodes, a prosthetic valve in 15%, and intravenous drug abuse in 8%. Viridans streptococci, *Staphylococcus aureus*, and nonviridans streptococcal and enterococcal species were implicated in 30%, 24%, 7%, and 6% of all episodes, respectively. Blood cultures were negative in 20% of all episodes. Cardiac surgery during treatment was performed in 49 (21.4%) of the episodes on median treatment day 7. Death during treatment or within 1 month after treatment was noted in 21 (9.2%) of the episodes on median treatment day 26.

ANTIMICROBIAL TREATMENT

A parenteral treatment course with a β -lactam antibiotic of 10 days or more was given 215 times for a mean of 29 days (95% confidence interval [CI], 22-30 days). Sequential treatment with 2 β -lactams for 10 days or more each occurred in several episodes (penicillin G, n = 16; cefuroxime, n = 18; cloxacillin, n = 8; ampicillin, n = 7; and piperacillin, n = 3). A change to another β -lactam drug was almost always due to an adverse event during the ini-

TEST PROCEDURE FOR EVALUATION OF POSSIBLE PENICILLIN ALLERGY

Prick Tests

Prick tests with solutions of benzylpenicillin sodium and penicillin v potassium at a concentration of 300 mg/mL as well as with penicilloyl-L-polylysine (PrePen) were performed on the volar surface of the forearm. Histamine hydrochloride at 10 mg/mL served as a positive control, and the test sites were inspected after 15 minutes. If no reaction was seen except for a typical wheal and flare at the site of the positive control, a challenge test was performed in 2 steps. One tenth of a therapeutic oral dose was initially given as a solution of penicillin V. If no reaction was reported or seen after 30 minutes, the test object received the additional part of the full dose. If this dose was also tolerated without problems within 1 hour, it was concluded that penicillin allergy with anaphylactic reactivity did not exist. A 24-hour follow-up for positive late reaction with a white blood cell count was also performed.

Antibody Determination

The occurrence of IgE antibodies to the benzylpenicilloyl determinant (BPO) was investigated using the Pharmacia CAP-Feia method (Pharmacia Diagnostics AB, Uppsala, Sweden).

Preparation of BPO-Coated Red Blood Cells. Human O-negative erythrocytes were washed 3 times and incubated as a 4% suspension in a 0.1-mol/L barbital buffer, pH 8.6, with 8% crystalline benzyl penicillin for 1 hour at 37°C and for 48 hours at 4°C. This procedure appears to covalently couple BPO groups directly to the red blood cell surface membrane.²¹

Passive Hemagglutination. Antibodies to BPO-coated human O-negative test erythrocytes were analyzed by

adding 50 μ L of a 2% suspension of test erythrocytes to 50 μ L of patient serum, 2-fold diluted with phosphate-buffered saline in 6 steps in microtiter wells. The microtiter plate was incubated for 1 hour at 37°C and at 4°C overnight before reading. Direct observation for an agglutination pattern of the test cell pellet was followed by mild shaking for further confirmation of agglutination. Agglutination was not considered BPO specific unless the reaction was inhibited by the addition of 50 μ L of penicillin solution left from the BPO coating to the patient serum before test erythrocytes were added.

ID-Gel Test. Agglutination of the test erythrocytes was also visualized using a gel test first described by Lapierre et al²² and Bromilow et al²³ and commercially developed into DiaMed MTC-ID by DiaMed AG, Cressie sur Morate, Switzerland, for simplified blood type testing. Briefly, a red blood cell suspension—in this case the BPO-coated human O-negative cells—was mixed with serum at the top of a gel column containing antihuman globulin and incubated for 1 hour at 37°C. The gel was centrifuged under standardized conditions (DiaMed MTC-ID Centrifuge). If agglutination occurred, the red blood cells were retained at the top of the gel, whereas an absence of agglutinating antibodies resulted in a red blood cell pellet at the bottom of the gel.

STATISTICAL METHODS

Differences between proportions were tested using the χ^2 test with Yates correction or Fisher exact test. Comparisons with respect to continuous variables were performed using the Wilcoxon signed rank test. Comparisons between antibiotic drugs with respect to incidence of adverse events were performed by the optimal test for comparisons of Poisson distributions.²⁴ A significance level of $P = .05$, 2-tailed test, was used in all tests.

tial β -lactam therapy. Penicillin G was given as a first-line β -lactam antibiotic in all 16 episodes. Cefuroxime was a first-line antibiotic in 4 of 18, cloxacillin in 4 of 8, ampicillin in 2 of 7, and piperacillin in 1 of 3 episodes.

Treatment courses with vancomycin of 10 days or more were given 51 times, in 21 episodes (41%) as a first-line antibiotic and in the remaining 30 episodes (59%) as a second-line antibiotic because of adverse reactions ($n = 16$) or treatment failures ($n = 14$).

Dosage regimens in the 266 episodes treated with a β -lactam or a vancomycin treatment course of 10 days or more are shown in **Table 1**.

ADVERSE REACTIONS TO β -LACTAM ANTIBIOTICS

A β -lactam treatment course of 1 day or more was started 292 times, and an adverse reaction requiring drug withdrawal within the first 9 days of treatment appeared in 8 episodes (3%). In 1 penicillin G-treated episode, a type I reaction occurred after the first injection, and in the other 7 episodes, the adverse events were rash ($n = 5$), fever ($n = 3$), or both.

Treatment courses with β -lactams of 10 days or more occurred 215 times, and drug-related adverse reactions,

as defined before, appeared overall in 70 episodes (33%). Frequency of adverse reactions and mean day of withdrawal of the different β -lactams and vancomycin involved in the adverse events are shown in **Table 2**. Frequency of drug-related adverse reactions varied from 11% to 51% for different β -lactam antibiotics. Mean duration of recurrent fever was 5 days (95% CI, 4-6 days), with rapid defervescence after withdrawal of the drug.

Neutropenia developed in a significantly higher frequency in penicillin G-treated episodes compared with cloxacillin-treated episodes (14% vs 4%; $P = .03$). No significant differences between the other β -lactam-treated groups could be noted. In 6 episodes, fever and/or neutropenia occurred with diarrhea ($n = 2$), thrombophlebitis ($n = 1$), or symptoms resembling serum sickness such as migrating arthralgia and/or abdominal pain ($n = 3$). In only another 4 episodes did other clinical manifestations considered to be drug related require withdrawal of β -lactam treatment.

Incidence of adverse events varied with treatment duration in the β -lactam-treated episodes (**Figure 1**). Increasingly higher frequencies were recorded during the treatment interval from day 15 to 30, declining to almost zero after this period despite continued β -lactam treatment in several episodes. Vancomycin-treated epi-

sodes had the same low incidence of adverse events throughout the treatment period, significantly lower compared with β -lactam-treated episodes. The risk of fever, rash, or neutropenia was 24 times higher during β -lactam treatment compared with vancomycin treatment. Episodes treated with penicillin G had a significantly higher incidence of adverse events compared with episodes treated with cloxacillin or cefuroxime (**Figure 2**). Similarly, episodes treated with ampicillin had significantly higher incidence of adverse events compared with cloxacillin-treated episodes.

Mean age for patients in whom an adverse reaction occurred during β -lactam treatment was significantly lower compared with mean age of patients without any adverse reactions (55 vs 63 years; $P = .003$), and mean estimated creatinine clearance²⁷ at admission was significantly higher (1.25 vs 1.03 mL/s [75 vs 62 mL/min]; $P = .009$). Mean age for patients with neutropenia, fever, and/or rash only or with no adverse reaction was 52, 56, and 63 years, respectively—a nonsignificant difference ($P = .17$). Adverse events occurred with the same frequency in episodes that fulfilled and did not fulfill the criteria of a final IE diagnosis (46% vs 42%).

SEQUENTIAL β -LACTAM ANTIBIOTIC TREATMENT AFTER AN INITIAL β -LACTAM ADVERSE REACTION

Adverse reactions that necessitated drug withdrawal occurred in 81 of the 292 episodes in which β -lactam treatment courses were started. A sequential treatment course

with a new β -lactam antibiotic (cefuroxime, $n = 17$; cloxacillin, $n = 2$; ampicillin, $n = 2$; or ceftazidime, $n = 1$) was given in 21 of these episodes for a mean of 17 days (95% CI, 6-39 days). Adverse reactions during the new β -lactam treatment courses were noted in 3 (14%) of 21 episodes after a mean of 10 days (95% CI, 8-12 days). Cefuroxime ($n = 2$) and ceftazidime ($n = 1$) were given in these 3 episodes.

The expected cefuroxime adverse event rate, based on the adverse event rate in the initially cefuroxime-treated episodes, was 0.017 events per treatment day after 10 days of treatment. The expected number of episodes of adverse events for patients receiving sequential cefuroxime treatment, based on the same figures, was 2, which exactly equaled the number of episodes that really did have an adverse event. Thus, the incidence of adverse events during cefuroxime treatment was the same, regardless of whether cefuroxime was given initially or after an adverse reaction to another β -lactam antibiotic.

INFLUENCE OF MDD ON ADVERSE DRUG REACTIONS

The MDDs of different β -lactams in episodes with and without any adverse reaction are presented in **Table 3**. The lowest MDD of penicillin G to induce neutropenia was 11.9 g/d given for 23 days. Penicillin G-treated patients who developed neutropenia, rash, or fever or who had no adverse reaction received an MDD of penicillin G of 14.8, 13.0, and 12.0 g/d, respectively. The difference in MDDs between episodes with fever and rash and no adverse reaction was significant ($P = .009$).

Results of 2 different MDD regimens of penicillin G are presented in **Table 4**. A significantly lower MDD of penicillin G was given during the later part of the study, and the incidence of neutropenia declined significantly compared with the first 2 years of the study. Other adverse reactions did not differ significantly between the 2 periods, however.

INFLUENCE OF TREATMENT DURATION ON NEUTROPENIC ADVERSE REACTIONS

The shortest treatment duration for a single β -lactam to induce neutropenia was 17 days in 2 episodes treated with cefuroxime and piperacillin. One patient treated with a combination of penicillin G and cloxacillin during the first 9 days developed neutropenia after 15 days of penicillin G treatment.

Table 1. Antibiotic Treatment Courses of 10 Days or More in Infective Endocarditis*

Antibiotic	No. of Courses	Duration of Therapy, Mean (95% CI), d	Daily Dose, Mean (95% CI), g/d	Total Dose, Mean (95% CI), g
Penicillin G	92	23 (21-24)	12.7 (12.2-13.3)	287 (265-308)
Cloxacillin	54	27 (24-30)	9.8 (9.2-10.4)	260 (231-290)
Cefuroxime	47	21 (18-24)	5.4 (4.9-5.9)	111 (95-128)
Ampicillin	18	23 (19-26)	11.3 (10.7-12.0)	255 (217-292)
Piperacillin	4	19 (10-31)	9.9 (5.3-14.6)	190 (56-325)
Vancomycin	51	26 (21-30)	1.5 (1.3-1.6)	37 (29-45)

*CI indicates confidence interval. For complete drug names, see "Patients and Methods" section of text.

Table 2. Adverse Events During Antibiotic Treatment Courses of 10 Days or More in Infective Endocarditis*

Antibiotic	Any Adverse Event, No (%)	Adverse Events, No (%)			Day of Drug Withdrawal During Treatment, Mean	Adverse Event Rate per Treatment Day ≥ 10 d
		Neutropenia	Rash	Fever		
Penicillin G (n = 92)	47 (51)	13 (14)	17 (18)	41 (45)	21	0.039
Cloxacillin (n = 54)	6 (11)	2 (4)	4 (7)	5 (9)	21	0.006
Cefuroxime (n = 47)	9 (17)	2 (4)	1 (2)	9 (17)	17	0.017
Ampicillin (n = 18)	7 (39)	1 (6)	4 (22)	4 (22)	20	0.030
Piperacillin (n = 4)	1 (25)	0	1 (25)	1 (25)	17	...
Subtotal β-Lactams (n = 215)	70 (33)	18 (8)	27 (13)	60 (28)	20	0.024
Vancomycin (n = 51)	2 (4)	0	1 (2)	2 (4)	17	0.001

*Ellipses indicate not applicable. For complete drug names, see "Patient and Methods" section of text.

NEUTROPHIL COUNT

As shown in **Figure 3**, neutrophil counts during 215 treatment courses with β -lactams decreased significantly every week during the first 3 weeks of treatment, during which no significant variations in neutrophil counts were observed in the 51 treatment courses with vancomycin, except in the second treatment week. Episodes treated with vancomycin had significantly lower neutrophil counts at the start of treatment compared with β -lactam-treated episodes (6.2 vs 8.5; $P = .03$), probably due to the fact that vancomycin was a second-line antibiotic in 59% of these episodes and was instigated after initial treatment of the acute infection and after adverse reactions to β -lactam antibiotics, including neutropenia. Analysis of first-line vancomycin-treated episodes showed a nonsignificantly higher neutrophil count of $7.3 \times 10^9/L$ at admission and no difference at all afterward compared with all vancomycin-treated episodes. The decline in neutrophil counts did not differ significantly between groups treated with different β -lactams, although the group receiving treatment with penicillin G had nonsignificantly lower neutrophil counts compared with other groups after 2, 3, and 4 weeks of treatment.

Episodes with neutropenia followed a uniform pattern of adverse events. After a mean of 21 days (95% CI, 18-23 days) of treatment, neutropenia developed in 18 episodes. Recurrent fever was noted in 16 (89%) of 18 such episodes and a rash in 5 (28%) of 18 of them. Mean duration of recurrent fever was 3 days (95% CI, 2-5 days), with rapid defervescence after withdrawal of the drug. Neutrophil counts normalized within 2 to 4 days, with the exception of 1 episode, in which penicillin G was given for at least 7 days to an afebrile patient in a severe neutropenic stage. The patient subsequently developed a recurrent fever that necessitated drug withdrawal, and total agranulocytosis without any superinfection lasted 10 days thereafter.

EOSINOPHIL COUNT

Serial eosinophil counts during 215 courses of treatment with β -lactams and 51 treatment courses with vancomycin are presented in **Figure 4**. A significant increase in eosinophils was noted during the first, third, and fourth weeks of β -lactam treatment, while vancomycin-treated episodes had no significant variations in eosinophils during treatment. Episodes treated with vancomycin had significantly higher eosinophil counts at the start of treatment compared with β -lactam-treated episodes (0.248 vs 0.101; $P = .04$), probably due to the fact that vancomycin was a second-line antibiotic in 59% of these episodes. A significant increase in eosinophil counts occurred in episodes treated with penicillin G as well as other β -lactams. The same degree of increase in eosinophil count occurred in episodes with adverse reactions as in those without (Figure 4).

SEROLOGICAL STUDIES

Serum samples collected 21 to 180 days after penicillin G treatment were available from 27 (57%) of 47 episodes with adverse reactions and from 19 (42%) of 45

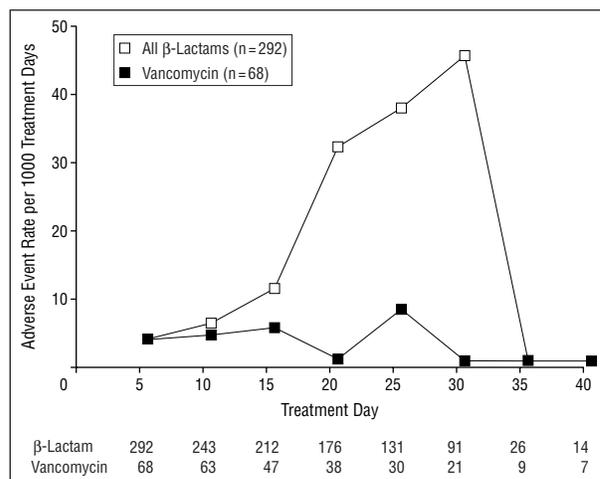


Figure 1. Rate of adverse events induced by parenteral β -lactams or vancomycin hydrochloride. Incidences per treatment day depending on time since start of treatment. For treatment courses with β -lactams vs vancomycin, $P < .001$, using the optimal test for comparisons of Poisson distributions.²⁴

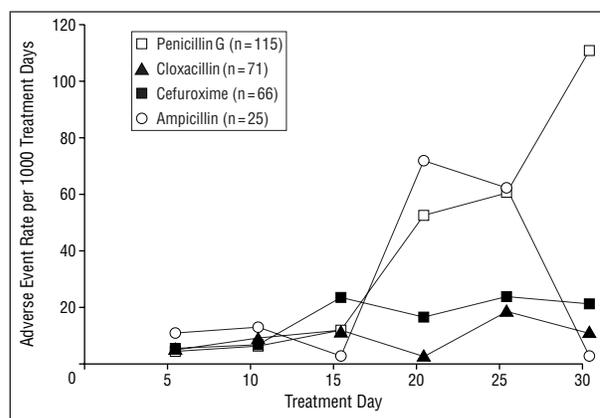


Figure 2. Rate of adverse events induced by different parenteral β -lactams. Incidences per treatment day depending on time since start of treatment. For treatment courses with penicillin G sodium vs cloxacillin sodium, $P < .001$; penicillin G vs cefuroxime sodium, $P < .001$; and cloxacillin vs ampicillin sodium, $P = .003$, using the optimal test for comparisons of Poisson distributions.²⁴

episodes without. The interval between penicillin G treatment and serum collection did not differ significantly between episodes with and without adverse reactions (57 vs 75 days; $P = .23$).

Results of the passive hemagglutination test and ID-gel test correlated well with each other ($r = 0.874$; $P < .001$).

PASSIVE HEMAGGLUTINATION

Two-fold dilutions of serum samples from penicillin G-treated patients were tested with red blood cells sensitized to penicillin G. A positive hemagglutination test result occurred significantly more often in serum samples from patients with adverse reactions compared with control serum samples from patients without any adverse reaction (16/27 vs 1/19; $P < .001$). Serum samples from episodes with isolated symptoms of fever, rash, or neutropenia were also positive. Serum samples with positive test results were drawn 21 to 150 days

Table 3. Mean Daily Doses of Different β -Lactam Antibiotics in Relation to β -Lactam-Induced Adverse Events During Treatment of Infective Endocarditis*

β -Lactam Antibiotic	Mean Daily Doses, g/d		P
	Adverse Events	No Adverse Events	
Penicillin G (n = 92)	13.5 (12.6-14.3)	12.0 (11.2-12.8)	.003
Cloxacillin (n = 54)	10.4 (8.4-12.3)	9.7 (9.0-10.4)	.04
Cefuroxime (n = 47)	6.6 (5.6-7.5)	5.7 (4.5-5.5)	.03
Ampicillin (n = 18)	11.1 (9.4-12.8)	11.5 (10.9-12.0)	.72
Piperacillin (n = 4)	12.0 (. . .)	9.2 (1.4-17.0)	. . .

*Data are given as mean (95% confidence interval [CI]). Ellipses indicate not applicable. For complete drug names, see "Patients and Methods" section of text.

Table 4. Effects on Incidence of Adverse Reactions of Reducing Daily Doses of Penicillin G

	1984-1985	1986-1988/ 1993-1995	P
Episodes	20	72	. . .
Mean daily dose, g/d	16.9	11.6	<.001
Mean duration of treatment, d	23	22	. . .
Adverse events, No. (%)			
Neutropenia	7 (35)	6 (8)	.01
Rash	5 (25)	13 (18)	.55
Fever	12 (60)	27 (38)	.37
Any adverse event	13 (65)	34 (47)	.27

*Ellipses indicate data not applicable.

after penicillin treatment was stopped. Results are shown in **Table 5**.

ID-GEL TEST

The ID-gel test for hemagglutinating penicillin antibodies also produced positive test results significantly more often in serum samples drawn from patients with adverse reactions compared with control serum samples from patients without any adverse reactions (16 of 27 vs 3 of 19; $P = .007$). Serum samples from episodes with isolated symptoms of fever, rash, or neutropenia were also positive. Serum samples with positive test results were drawn 21 to 150 days after penicillin treatment was stopped (Table 5).

CAP-Feia IgE ANTIBODY TEST

A follow-up study was performed a mean 6.2 years (95% CI, 0.2-12 years) after treatment in 22 of 47 patients who had experienced an adverse reaction to penicillin G. No IgE antibodies against penicillin G or penicillin V could be found using a CAP-Feia test, with the exception of 1 patient who was tested only 2 months after treatment.

PENICILLIN CHALLENGE

A new course of treatment with oral or, in a few patients, parenteral penicillin was given to 13 (59%) of 22 patients during a mean follow-up of 6.2 years (95%

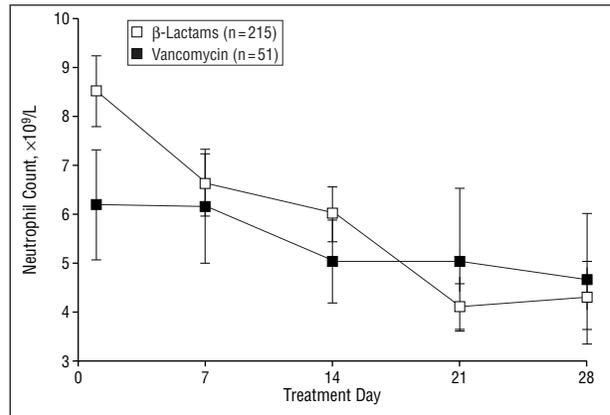


Figure 3. Serial neutrophil counts in 215 β -lactam and 51 vancomycin hydrochloride treatment courses of 10 days or more in infective endocarditis. Bars indicate 95% confidence intervals. β -Lactam treatment courses: day 0 vs 7, $P < .001$; day 7 vs 14, $P < .001$, and day 14 vs 21, $P < .001$. Vancomycin treatment courses: day 7 vs 14, $P = .006$.

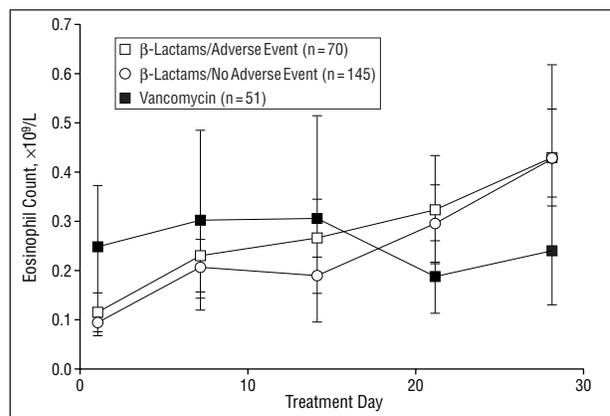


Figure 4. Serial eosinophil counts in 215 β -lactam treatment courses of 10 days or more with respect to adverse or no adverse events and in 51 vancomycin hydrochloride treatment courses of 10 days or more in infective endocarditis. Bars indicate 95% confidence intervals. β -Lactam treatment courses: day 0 vs 7, $P < .001$; day 14 vs 21, $P = .001$; and day 21 vs 28, $P = .02$. Vancomycin treatment courses vs β -lactam treatment courses at start of treatment, $P = .04$.

CI, 0.2-12 years). The new treatment periods were all of short duration, and no adverse events occurred. The 9 remaining patients, including the patient with an IgE response, underwent prick tests with penicillin G, penicillin V, and penicilloyl-polylysins without any adverse reactions. With the exception of the IgE-positive patient, they were all subsequently challenged with a therapeutic oral dose of penicillin V without any complications.

COMMENT

The risk of adverse events with β -lactam antibiotics used for IE is mainly dependent on the length of treatment. Thus, in this study, very few adverse effects were observed during the first 10 days of treatment. One type I reaction occurred in 292 given courses of treatment with β -lactam drugs, giving a rate of 0.3%—or 0.9% of 115 courses of penicillin G. The expected rate of type I reactions is reported to be 0.004% to 0.2% with penicillin G^{28,29} and even less with cephalosporins.^{30,31}

Table 5. Antibody Assays in Penicillin G–Treated Patients

Positive	Adverse Reaction (n = 27)	No Adverse Reaction (n = 19)	P*
Passive hemagglutination			
1:2	5	1] <.001
1:4	3	0	
1:8	6	0	
1:16	2	0	
Total, No. (%)	16 (59)	1 (5)	
ID-gel			
1+	2	3] .007
2+	2	0	
3+	6	0	
4+	6	0	
Total, No. (%)	16 (59)	3 (16)	

*Using Fisher exact test.

After 10 or more days of treatment with β -lactams, delayed adverse events were common and appeared in 33% of all treatment courses after a mean of 20 treatment days. The same pattern of increasingly higher frequencies of adverse events during the treatment interval of up to 30 days appeared in all β -lactam treatment courses. After 30 days of treatment, the incidences declined to almost zero, possibly due to halted β -lactam treatment in patients sensitive to the adverse effects. Neutropenia was never observed before 17 days of treatment with a single β -lactam. Single events of fever or rash could be noted during the whole treatment period, however. Cutaneous reactions or fever occurred about 5 times more frequently compared with incidences reported from several other studies of β -lactam treatment of varying duration.³²⁻³⁵ In only 4 treatment courses (2%) did other drug-related adverse effects, such as diarrhea or thrombophlebitis, lead to drug withdrawals. After withdrawal of the drugs, the adverse events, including neutropenia, disappeared quickly.

In this study, it was possible to estimate the risk of delayed hypersensitivity reactions with 4 different β -lactams: penicillin G, ampicillin, cefuroxime, and cloxacillin. Such calculations may not have been carried out earlier. Penicillin G treatment seemed, with the MDDs given, to have a more than 6 times higher risk of inducing an event of fever, rash, or neutropenia compared with cloxacillin, the drug with the least risk. The reason for this great difference must be considered unknown. Vancomycin induced adverse events in 2 treatment courses only, and no similar symptoms as with β -lactams occurred. Long-term vancomycin treatment implied a 6- to 39-times lower risk of adverse events in comparison with different β -lactams. Treatment with vancomycin was, of course, well controlled, with measurement of serum concentrations and serum creatinine to avoid toxic effects.

Which mechanisms are involved in the frequent delayed adverse reactions to β -lactams? The described adverse reactions were to some extent related to dosage. The daily dose in episodes with adverse events was significantly greater for penicillin G, cloxacillin, and cefuroxime in comparison with episodes without. Neutropenia occurred significantly more often in high-dose com-

pared with lower-dose penicillin G–treated episodes (35% vs 8%). Other types of adverse reactions did not differ significantly. This suggests that 2 different pathogenetic mechanisms may be involved in neutropenic adverse reactions.

We used 2 different serological methods to study hemagglutinating antibodies to penicillin in serum samples drawn 21 to 180 days after penicillin G treatment was stopped. The passive hemagglutination test and ID-gel test correlated well to each other, and both results were positive in a significantly higher percentage of episodes with an adverse event compared with episodes without. Penicillin antibodies could be demonstrated by both methods as long as 150 days after treatment in a single episode. In an early study by Radermecker and Salmon,³⁶ a hemagglutination test result was positive in 71% of patients recently sensitized to penicillin compared with 16% of penicillin-treated nonallergic patients. Sanders et al⁴ found hemagglutinating antibodies to penicillin G 1 month after treatment in 22 of 24 subjects, all of whom had adverse reactions to cephalothin sodium or cephapirin sodium treatment.

It has been proposed by Levine et al³⁷ that parenteral penicillin therapy always induces a transient IgM antibody response. Our serological antibody finding was a weak direct agglutination of BPO-conjugated red blood cells when tested in the wells of a microtiter plate. A clearly visible reaction, however, was seen when test cells and positive serum samples were centrifuged through an antihuman globulin-containing gel (ID-gel test). This would indicate that the antibodies were of the IgG type, produced as a result of a stronger immune response than expected to follow ordinary β -lactam treatment.

The significant correlation between these antibodies to the BPO radical and delayed adverse reactions observed during β -lactam treatment favors the hypothesis that an immune mechanism is involved. Metabolites of penicillin, particularly the BPO radical, have haptenic properties and may conjugate to various proteins and cells. Drug-specific antibodies to penicilloylated neutrophils could explain β -lactam-induced neutropenia by a mechanism similar to that reported to occur in penicillin-induced immune hemolytic anemia.^{14,38-43}

It is suggested that the antigenicity of penicillin preparations is caused by giving penicillin G solutions that are not freshly prepared, allowing degradation products to form, which gives rise to antigenically active metabolites.⁴⁴ If so, the use of freshly prepared solutions would reduce all kinds of immune-mediated adverse reactions to penicillin. In our study, however, only freshly prepared solutions were given. It has also been proposed that antigenicity is caused by impurities and preformed metabolites.⁴⁵ In that case, the use of purer preparations would reduce all kinds of immune-mediated adverse reactions to penicillin. It seems likely, however, that BPO conjugation to white or red cells of the blood results from metabolite production in vivo, particularly when higher doses are given. If so, the adverse reactions described in this article would not be expected to be reduced in frequency by the use of purer preparations.

A general decline in neutrophils was seen during β -lactam treatment. During the first week of treatment, such

a decline could be explained by eradication of a sepsis syndrome. In the β -lactam-treated episodes reported herein, a highly significant decline continued to occur during 3 weeks of treatment and was most pronounced during the third week. This decline was noted for all β -lactams, although most pronounced in the penicillin G-treated episodes, which also generated the highest frequency of adverse events. Vancomycin-treated episodes, on the other hand, showed a very moderate and nonsignificant decline during the treatment period. We also observed in a few cases that sequential or combinations of β -lactams for treatment induced an earlier onset of neutropenia.

After demonstrating a dose-dependent inhibition of growth of neutrophil colonies in vitro for different β -lactams, Neftel et al^{1,46} postulated a direct toxic effect on neutrophil progenitors. They questioned the relevance of antibodies directed against penicillin and penicilloylated cells, due to the findings of circulating IgG antibodies to penicillin after treatment with penicillin G, ampicillin, floxacillin sodium, or piperacillin, irrespective of whether neutropenia developed.^{2,47} Obviously, these findings are different from ours.

Both types of mechanisms might be involved in the neutropenic reaction, a hypothesis that has also been proposed by Murphy et al.⁴⁸ A β -lactam-induced immune-mediated reaction might well give rise to various clinical manifestations such as fever, rash, or depression of the neutrophils. The dose-related toxic effect of β -lactams may compromise the ability of bone marrow to compensate for the immune destruction of neutrophils, which influences the severity of neutropenia. The extent of adverse events and involvement of immune-mediated or toxic reactions may vary between patients.

In agreement with previous reports from studies of adverse events during penicillin treatment,^{49,50} elderly patients in our series had a lower frequency of adverse reactions to β -lactams compared with younger adults.

Eosinophilia has been associated with adverse events in numerous reports. In our treatment series, β -lactams induced higher eosinophil counts, irrespective of whether any adverse reaction occurred. The increase in eosinophils during β -lactam treatment might be due to a healing process, an immunologic reaction to the β -lactam antibiotic itself, or its degradation products not associated with any adverse reaction. Eosinophilia is an old clinical observation during penicillin treatment of a streptococcal infection not associated with any adverse reaction.⁵¹ The absence of eosinophilia during vancomycin treatment also supports the assumption that immunologic reaction to β -lactams occurs during treatment.

In conclusion, we could significantly correlate the presence of BPO antibodies to the occurrence of delayed adverse events, characterized by recurrent fever, rash, or neutropenia. The correlation between dose and duration of treatment on the one hand and adverse reactions on the other supports the assumption of a dose-related toxic trigger mechanism enhanced by an immunologic reaction.

Of clinical importance is the fact that this immune reaction is not mediated by IgE antibodies. β -Lactams may be used once again, but if treatment lasts more than 2 weeks, close surveillance of neutrophils is indicated.

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Correction

Error in Byline. In the article by Chen et al titled "Efficacy of Ondansetron and Prochlorperazine for the Prevention of Postoperative Nausea and Vomiting After Total Hip Replacement or Total Knee Replacement Procedures: A Randomized, Double-blind, Comparative Trial," published in the October 26 issue of the ARCHIVES (1998;158:2124-2128), the name of one of the authors was misspelled in the byline on page 2124. The byline should have read as follows: "Jack J. Chen, PharmD; David G. Frame, PharmD; T. Jeffrey White, PharmD."