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Neutropenia due to β lactamine antibodies

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Abstract

Neutropenia developed in 13 patients during treatment with β lactamines. The time of onset ranged from eight to 27 days after beginning treatment and occurred with doses as low as 40 mg/24 hours. Concomitant symptoms were eosinophilia, rashes, and fever. Leucoagglutinins were detected in eight out of nine patients by the microleucoagglutination technique.

Clinical and serological findings suggested that neutrophils become sensitised as a result of absorption on the cell membrane of drug-antibody immune complexes. An immune mediated pathogenesis for neutropenia induced by β lactamine seems highly probable.

Introduction

Neutropenia induced by β lactamine has been reported in about 150 patients since 1960.[1 2] Symptoms are never severe, and the neutrophil count quickly returns to normal once treatment is stopped. The basic pathogenic mechanism of the complication is not fully understood, but most reports postulate either a toxic effect or dose related damage[3-7]; an immunologically mediated mechanism has rarely been implicated[8 9] owing to the absence of demonstrable drug dependent leucoagglutinins.[10]

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We report 13 patients who developed neutropenia after receiving intravenous or oral β lactamines. Leucoagglutinins were detected in eight out of nine patients tested.

Patients

Table I summarises the 13 cases of neutropenia, which occurred during treatment with penicillin, oxacillin, amoxycillin, ampicillin, cefoperazone, cefotaxime, and ceftazidime. These β lactamines were administered either intravenously (10 cases) or by mouth (three cases) at a dose of 40-250 mg/kg/24 hours for severe sepsis. None of the patients had a history of allergy to penicillin. Other medications administered concomitantly varied, apart from an associated aminoglucoside (streptomycin, gentamicin, or amikacin). We excluded these as offending agents, since they were continued during the episode of leucopenia. In case 10 neutropenia was noted for the first time after oxacillin. Recovery was rapid after withdrawal of this drug, and the patient was given cephazolin. The neutrophil count fell again (to 88×10^6/l) and rapidly returned to normal once the drug was stopped.

Neutropenia was observed as early as day 8 after starting treatment in one patient, the longest latent period being 27 days. The mean time of onset after starting treatment was 19 days. Neutrophil counts ranged from 24 to 1885×10^6/l. Total white cell count was less than 3300×10^6/l in all but one patient, in whom there was a dissociation between absolute neutrophil count (80×10^6/l) and leucocyte count (8000×10^6/l). There was no depression in red blood cell series.

Associated eosinophilia ($> 400 \times 10^6$/l) was observed in six patients, thrombocytopenia in one, and blood myelocytosis in two. A transient generalised rash occurred in one patient and fever in three. Renal and hepatic function remained normal in all patients. The neutrophil count returned to normal within one to six days after stopping the β lactamines alone.

One patient (case 13) consented to receive a low dose of the same β lactamine (amoxycillin 500 mg by mouth) after her white cell count had returned to normal. This patient experienced a recurrence of the leucopenia, beginning within the next 24 hours.

The bone marrow was examined in six patients. The results showed

arrested maturation with a lack of granulocytes in three, complete absence of mature neutrophils in one, hyperactive myelopoiesis in one, and an increased eosinophil content in one.

Materials and methods

Leucoagglutinins were detected using the leucocyte microagglutination test. Normal leucocytes were isolated from 10 ml venous blood samples with 1 ml 10% edetic acid. This was then diluted 1/1 with 50% dextran (dextran T500, Pharmacia, Sweden) followed by sedimentation at room temperature for 15 minutes. The supernatant rich in leucocytes was aspirated and centrifuged at 250 *g* for 10 minutes. The leucocyte pellet was washed three times in Ham's medium (Eurobio, France). The contaminating erythrocytes were lysed with 0·83% NH_4Cl for 15 minutes. Cell viability (trypan blue exclusion) was greater than 90%. Polymorphs constituted 78% of the final cell suspension, the rest being lymphocytes and monocytes. The final cell suspension was adjusted to yield a granulocyte count of 16×10^9/l.

The leucocyte microagglutination test was performed in triplicate by mixing equal volumes (20 µl) of the patient's serum, leucocytes (4000/µl) from a normal donor (blood group O), and the drug antigen in a flat bottomed microtitre plate (Falcon). Sera collected from a patient at different times were tested against the same donor neutrophils.

The drugs used by the patients (table I) were utilised as antigens. They were obtained from commercial sources in France. All drug antigens were dissolved in Ham's medium.

The plate was incubated at 37 C for three hours and examined at intervals for leucoagglutination using the low power objective of an inverted phase microscope. The reactions were read blind by two different observers and graded arbitrarily as 1+ (<25% of cells agglutinated), 2+ (25% of cells agglutinated), 3+ (50% of cells agglutinated), and 4+ (>90% of cells agglutinated).

Blood samples were taken from the patients at various intervals and the sera stored at −60 C before use. Doubling dilutions of sera starting from 1/1 to 1/16 were used. Two concentrations of the drug antigen were utilised (100 and 500 IU/ml).

Three control tests were performed for each assay. In control test A we used the patient's serum, normal human leucocytes, and saline instead of β lactamines (serum control). This control ruled out leucoagglutination due to isoantibodies. Control test B used compatible normal human leucocytes, fresh compatible human serum, β lactamine solution, and the patient's serum after recovery (drug antigen control). Sera from the patients were tested at least twice, during acute leucopenia and during convalescence. In control test C we evaluated the possible role of the immune complex(es) by dialysing the serum against low pH buffer (0·1M acetate buffer, pH 4). This was done to dissociate the complexes, and the assay was performed as described above.

Results

Table II gives the anti-β lactamine antibody titres. Anti-β lactamine antibodies were detected in eight of the nine patients tested. The antibodies were present only in serum specimens collected within the first two days after the onset of neutropenia and not in those collected after complete recovery. Some patients had a low antibody titre in their serum when it was not diluted. The antibody titre in a patient's serum varied with the use of different donor neutrophils but the results were still clear cut.

Antibody activity was not detected in the serum in case 12. This was the only sample where the test was done with serum collected on day 5 after stopping treatment when the neutrophil counts were still low.

Table III shows the kinetics of the antibody after challenging a patient with the same β lactamine. Antibodies were detectable for up to three days after challenge with the drug, while the neutrophil count returned to normal two days later. Drug induced leucoagglutinins were detected at high serum dilution even in the absence of β lactamine on day 1 after its withdrawal. When using serum from day 2 or day 3 agglutination was more easily detected by adding the drug antigen.

Additional studies were carried out to investigate the possible role of immune complexes and complement in the drug reaction. Table IV

TABLE II—*β Lactamine antibody titres in nine patients tested with leucocyte microagglutination technique. Results for each patient obtained with serum at nadir of neutropenia (a) and serum after complete recovery (b)*

Case No		Dilution of serum				
		1/1	1/2	1/4	1/8	1/16
2	(a)	2+	2+	1+	1+	1+
	(b)	0	0	0	0	0
3	(a)	2+	2+	2+	1+	1+
	(b)	0	0	0	0	0
4	(a)	1+	1+	1+	1+	0
	(b)	0	0	0	0	0
6	(a)	1+	1+	2+	2+	2+
	(b)	0	0	0	0	0
7	(a)	0	0	1+	2+	2+
	(b)	0	0	0	0	0
8	(a)	2+	2+	2+	2+	2+
	(b)	0	0	0	0	0
11	(a)	0	1+	1+	1+	1+
	(b)	0	0	0	0	0
12*	(a)	0	0	0	0	0
	(b)	0	0	0	0	0
13	(a)	4+	4+	4+	4+	4+
	(b)	0	0	0	0	0

*Serum obtained five days after stopping treatment.

TABLE III—*Relation of leucocyte microagglutination and neutropenia in case 13 after challenging with amoxycillin (10 mg/kg)*

Time after challenge (days)	Neutrophils ($\times 10^6$/l)	Leucocyte microagglutination test			
		Without drug antigen		With drug antigen	
		Cells agglutinated	Titre	Cells agglutinated	Titre
0	6734	0		0	
1	2414	4+	1/16	4+	1/16
2	2204	1+	1/4	4+	1/8
3	2788	1+	1/1	2+	1/4
4	2898	0		0	
5	4995	0		0	

TABLE I—*Details of 13 cases of penicillin induced neutropenia*

Case No	Age and sex	Diagnosis	Antibiotic, dose (mg/kg/day), and route	Time of onset of neutropenia after start of treatment (days)	Leucocyte profile ($\times 10^6$/l) at nadir of neutropenia (total white blood cells/ neutrophil count) (%)	Time of onset of recovery after end of treatment (days)
1	57 F	*Pseudomonas aeruginosa* mediastinitis	Cefoperazone, 70, IV	21	2900/1885 (65)	2
2	46 M	Endocarditis	Penicillin, 250, IV	25	2400/24 (1)	4
3	46 M	Enterobacter sternitis	Cefotaxime, 66, IV	27	2900/870 (30)	1
4	19 M	Staphylococcal mediastinitis	Oxacillin, 160, IV	21	2200/990 (45)	1
5	24 M	Staphylococcal endocarditis	Ceftazidime, 72, IV	8	2300/736 (32)	1
6	32 M	Staphylococcal endocarditis	Oxacillin, 100, IV	25	2000/180 (9)	2
7	59 F	Sepsis	Cefotaxime, 40, IV	10	1300/500 (38)	3
8	46 M	Sepsis	Ampicillin, 200, IV	20	1500/315 (21)	6
9	1 F	Streptococcal sepsis	Amoxycillin, 50, by mouth	23	8000/80 (1)	3
10	54 M	Staphylococcal mediastinitis	Oxacillin, 150, IV.	18	2600/702 (27)	1
			Cephazolin, 100, IV	1	2000/88 (4)	1
11	33 F	Staphylococcal endocarditis	Oxacillin, 116, IV	20	2900/180 (6)	1
12	23 M	Streptococcal cerebral empyema	Amoxycillin, 58, by mouth	21	3300/264 (8)	1
13	27 F	Typhoid	Amoxycillin, 50, by mouth	13	1470	1

IV = Intravenously.

TABLE IV—*Experiments showing possible role of immune complexes in neutropenia*

Case No	Leucocyte microagglutination test		
	Fresh serum from patient	Low pH dialysed serum from patient	Low pH dialysed serum from patient plus β lactamine solution
2	2+	0	1+
6	3+	0	2+
8	2+	0	2+
13	4+	0	3+

shows that after a low pH dialysis which dissociated the immune complexes the patients' sera lacked antibody activity. This returned when the drug antigen solution was added.

Discussion

Isolated neutropenia occurring as a side effect of treatment with β lactamine has been described in about 150 patients over the past 20 years. The mechanism of the disorder—that is, toxic or immune mediated or both—is not fully understood. Our study suggests an immune mediated pathogenesis, based on the clinical manifestation of leucopenia and the results of immunological tests for antibodies.

The following principal clinical features may be cited. Firstly, neutropenia occurs with penicillin G or its semisynthetic derivatives; cross reactivity, which is not well documented,[11-13] apparently occurred in our case 10. Secondly, intravenous administration does not appear to be a factor[6] and we observed neutropenia in three patients after oral treatment. Thirdly, neutropenia may be detectable by the eighth day of treatment, although the latent period may be longer.[11] In our series the neutrophil count returned to normal within one to six days after withdrawal of the β lactamine alone (table I). Fourthly, concomitant signs include eosinophilia, fever, and maculopapular rash,[9 13] which may occur as a result of antibodies combining with protein antigens. Finally, in our patients neutropenia occurred at a dose as low as 40 mg/kg/24 hours; in most other reported series these reactions to β lactamines were related to a higher dose.[6 7 9 10] A high serum concentration of β lactamine probably favours its binding to blood proteins to become antigenic.[16]

Examination of the bone marrow usually shows hyperactive myelopoiesis with no mature neutrophils (arrested maturation) and occasionally with an increased eosinophil content. A concomitant depression of other stem cells seems very rare. This granulocytic hypoplasia is difficult to explain but may be due to an immune suppression of granulopoiesis.[17 18] Although bone marrow hypercellularity usually occurs during the very early and recovery phases of an immune agranulocytosis, an intermediate phase of bone marrow hypercellularity may sometimes be seen.[14]

Our immunological data strongly suggest that leucopenia was due to a serum factor which agglutinated leucocytes. Several in vitro serological techniques have been used to study drug related, immune mediated destruction of mature cells,[19] but as yet there is no generally agreed method for detecting neutropenia. Tests may detect different antibodies and vary in their clinical significance—for example, indirect immunofluorescence has been proposed for the detection of isoantibodies.[20] In our assay the presence of drug was not required when the serum was obtained shortly after stopping the drug, though agglutination was more easily detectable in the presence of the drug thereafter (table III). The possibility of the antibody being bound to the neutrophil,[20] however, has not been checked.

Anti-β lactamine antibodies were detected in the sera of eight out of nine patients tested, including one in whom antibodies were found after challenge. That antibodies are not detected more frequently may be explained by the speed of degradation of antibodies, especially those of high affinity and low concentration. Hence it is important to test serum taken on day 1 after stopping treatment. This was shown in case 12, where no antibodies were found in the serum from day 5 after stopping the drug. Moreover, the antibody titres in our patients were low and demonstrable for only a few days after exposure to the antigen.

The agglutinating activity in the sera was either abolished or decreased by treatment with low pH dialysis and restored by the addition of the antigen. These observations are in keeping with drug induced blood dyscrasias caused by immune complexes of drug and antibody.[21 22]

Among the many reports of penicillin induced neutropenia only three have suggested an immunological mechanism whereby there occurs an intimate association of drug with a target cell that is subsequently damaged as a result of the binding antibody —namely, opsonising antineutrophil antibodies as reported in four patients,[8] which were dependent on the drug concentration in the serum but independent of complement; anticephalosporin antibodies reacting with coated neutrophils as reported in one case[13]; and antineutrophil antibodies in four patients.[9]

Our findings suggest that the leucopenia in our patients was immunologically mediated. β Lactamine associated antibodies already formed in a patient's serum may agglutinate leucocytes by a complement dependent mechanism. Our study also shows that neutrophil counts should be regularly checked during allergic reactions to penicillin.

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