

Allergological Examination of Cystic Fibrosis Patients with Skin Reactions During Carbenicillin Treatment

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Sixteen of 84 cystic fibrosis patients developed skin manifestations during intensive carbenicillin therapy. The possibility of allergic drug reactions was examined. None of the patients showed any significant levels of antibodies of IgE, IgG or IgM classes against carbenicillin and different penicilloyl determinants as recorded with RAST, sandwich-radioimmunoassay and double antibody assay. The leukocytes of the patients did not release histamine on *in vitro* provocation with carbenicillin. Furthermore, none of the patients responded to prick test or to peroral penicillin provocation. The negative findings indicated that the recorded reactions were most probably not due to antibody-mediated allergy of type I or type III.

Key words: adverse drug reactions; carbenicillin; cystic fibrosis; double antibody assay; histamine release; penicillins; RAST; sandwich-radioimmunoassay; skin test.

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During treatment with carbenicillin of *Pseudomonas aeruginosa* infection of the lungs, 16 cystic fibrosis patients developed skin manifestations, usually described as allergic reactions to penicillin. To obtain information regarding the possibility of allergic drug reactions, the patients' sera were tested for antibodies of the IgE as well as of the IgG and IgM classes against carbenicillin and different penicilloyl-human serum albumin conjugates. The IgE antibodies were analyzed with RAST and with histamine release from peripheral leukocytes. The sera were further tested for the presence of penicilloylated antigens. In addition, conventional skin test and peroral provocation with phenoxymethylpenicillin were performed.

PATIENTS

Eighty-four cystic fibrosis patients were for several years treated partly with intensive

carbenicillin therapy (see below) administered three to four times yearly during 14-day periods and partly with other penicillins. During a period with carbenicillin treatment 16 patients developed skin manifestations in spite of the fact that all patients had previously been treated with the same regimen without adverse effects. The skin reactions were of such magnitude that the administration of carbenicillin was discontinued. The carbenicillin was infused intravenously over a 10-min period three times a day. The total daily dose was 0.5 g per kg. The symptoms, the duration of treatment before the symptoms, and the interval between the last infusion and the start of the symptoms are recorded in Table 1. The patients had no atopic diseases, except for patients No. 3 with food allergy and No. 13 with grass pollen hay fever (Table 1) and none of them showed drug reactions caused by the administration of other drugs than penicillins during several years.

Table 1

Adverse reactions to carbenicillin. Symptoms, duration of carbenicillin treatment and interval between last infusion and onset of symptoms are given

Patient No.	Periods ^a (nos.)	Symptoms ^b						Duration, days	Interval	Serum ^c assay	Histamine ^c release
		Q	U	E	P	F	A				
1	1			+	+	+	+	8	15 min	A	D
2	3			+				7	1 h		D
3	2			+				10	1 min		D
4	2				+	+		0	2 h		D
5	5			+		+		4	5 h		N.D.
6	1				+			0	1 h ^d		D
7	1	+						0	1 min ^e		D
8	1			+	+			8	1 h		C
9	6				+			0	10 min		A
10	3			+	+	+		6	3 h		A
					+	+		6	4 h		C
11	1			+	+	+		0	5 h	B	N.D.
12	2			+		+		8	4 h		D
13	2	+						3	6 h		D
14	6				+	+		8	10 min		D
15	1			+	+	+		0	2 h	C	N.D.
16	4			+		+		3	3 h	D	D

^a Number of periods with carbenicillin treatment before onset of adverse reactions.

^b Q = Quincke's edema; U = urticaria; E = exanthema; P = prurigo; F = fever; A = arthralgia.

^c Time of serum assay and histamine release after onset of carbenicillin reaction: A: 2-7 days; B: 8-30 days; C: 1-6 months; D: 0.5-2 years.

^d First infusion.

^e Second infusion.

N.D.: not done.

METHODS

Prick tests were performed with carbenicillin (0.1 g/ml) and with histamine and physiologic saline as controls. The patients were observed 1 day in the ward and questioned for later reactions.

Penicillin provocation. If negative in the skin tests, the patients were provoked orally by phenoxymethylpenicillin, 0.25 MIE the first day, the next day 0.5 MIE, and if tolerated, a full dose was given.

Histamine release from leukocytes. By venipuncture 10 ml blood samples were collected in heparinized plastic tubes. Peripheral blood leukocytes with approximately 2% basophils

were isolated by the Ficoll-Hypaque gradient method (9) and 200 μ l of the leukocyte suspensions were incubated in plastic tubes at 37°C for 40 min with 10 μ l carbenicillin or benzylpenicilloylated human serum albumin (Astra Läkemedel AB, Södertälje, Sweden) in final concentrations from 10^{-4} - 10^{-1} mg/ml, and the release of histamine was estimated by a spectrofluorometric assay (10).

Radioallergosorbent test (RAST). The presence of IgE antibodies against penicilloyl determinants derived from various penicillins was analyzed by the RAST method, originally described by Wide et al. (11). The commercial kit (Pharmacia Diagnostics Ltd., Uppsala, Sweden) was used as described by the manufacturer for tests of reagents against

benzylpenicilloyl and phenoxymethylpenicilloyl. In the tests these determinants had been conjugated to human serum albumin, which was attached on paper discs through CNBr linkage. Carbenicilloyl-human serum albumin conjugates fixed to microcrystalline cellulose (4) were used to record IgE antibodies with side chain specificity for carbenicillin. In order to detect any IgE antibodies induced by impurities possibly occurring in the carbenicillin preparations, ampicilloyl-*E. coli* acylase, prepared as described by Parker (8) and benzylpenicillin fermentation products were conjugated to CNBr-activated microcrystalline cellulose. The test procedure described by Pharmacia Diagnostics Ltd. (Uppsala, Sweden) was followed when running the tests. Radio-iodinated anti-IgE was purchased from Pharmacia Diagnostics Ltd. and in all tests used according to the manufacturer's description. The results were expressed as described by the manufacturer for the analysis of IgE antibodies against benzylpenicilloyl and phenoxymethylpenicilloyl determinants. The other values were compared with serum from non-atopic individuals used as controls (4).

Assays of IgG and IgM penicilloyl antibodies. Total penicilloyl antibody levels were recorded with a sandwich-radioimmunoassay as described by Ahlstedt et al. (2). In this assay, azidocilloylated human serum albumin with 12 determinants fixed to microcrystalline cellulose reacted with the antibodies in the test serum during a 16-hour incubation. For radioactive counting, radio-iodinated human serum albumin conjugated with ampicillin (5) was added and the procedure was continued as described by Ahlstedt et al. (1). The double antibody assay was performed as described by Kristofferson et al. (6).

Assay of penicilloylated antigen in patient serum. The radioimmunoassay, RIA (5), was employed according to Ahlstedt et al. (2) to determine any penicilloylated antigens in the patient serum. Thus, 4-, 8-, 16-, and 32-fold

dilutions of patient serum were added to the immunosorbent carrying penicilloyl-specific rabbit antibodies. These were allowed to react with the serum, possibly penicilloylated *in vivo* during the therapy in competition with radio-iodinated human serum albumin conjugated with ampicillin. The results were expressed in relation to reference concentrations of human serum albumin conjugated with 14 azidocilloyl determinants (5) and given in ng of antigen per ml.

RESULTS

No positive results of IgE, IgG and IgM antibody levels were recorded with any of the antigens. Two patients (Nos. 1 and 5) showed some levels of penicilloylated antigens in their sera, corresponding to about 700 ng and 500 ng of azidocilloyl-human serum albumin per ml of serum. In the histamine release assay all tests were negative, showing the absence of carbenicillin-specific IgE bound to the basophil leukocytes. Leukocytes from three patients (Nos. 1, 3 and 6) were also challenged with benzylpenicilloylated human serum albumin but no histamine release could be recorded.

All patients showed negative prick tests and none of them responded to peroral provocation with phenoxymethylpenicillin. In spite of the negative *in vivo* and *in vitro* tests, but due to the serious carbenicillin skin manifestations (Table 1) the treatment was continued with other penicillins. All the patients tolerated phenoxymethylpenicillin and 12 of 13 patients tolerated ampicillin, both given *per os*. Furthermore, two of two patients tolerated carbenicillin, three of five tolerated Azlocillin* and one of three tolerated Piperacillin*, all of which were given intravenously. One patient reacted with ampicillin flush (No. 15) and three patients reacted with a serum sickness picture during treatment with Piperacillin (Nos. 2 and 14) and with Azlocillin (Nos. 2 and 7).

DISCUSSION

This investigation comprised 16 patients with cystic fibrosis who developed skin manifesta-

tions during carbenicillin treatment for pulmonary *Pseudomonas aeruginosa* infections. Since the skin manifestations had a resemblance to allergic drug reactions, the patients were examined for antibodies of IgE, IgG, and IgM classes against carbenicillin and different penicilloyl determinants suspected to be responsible for the drug reactions. However, none of the patients showed any significant level of drug-specific antibodies determined by RAST, sandwich-radioimmunoassay and double antibody assay. Furthermore, none of the patients released histamine on *in vitro* provocation of their basophil leukocytes with carbenicillin. All skin tests were negative and the same applied to the peroral penicillin provocation.

The finding of penicilloylated antigens in serum of two patients without any antibody that could interfere with the test, corresponds to previous findings of such antigen in a few cases treated with high doses of penicillin (3, 7). The finding might indicate an *in vivo* penicilloylation of human proteins, but antibodies of IgE, IgG and IgM classes against such penicilloyl compounds could not be demonstrated in the sera.

All the negative findings indicate that the reactions seen during carbenicillin treatment were most probably due neither to allergic type I nor to type III reactions. Other adverse drug reactions must therefore be taken into account such as type IV (cell-mediated) reactions or reactions which do not depend on immunological mechanisms, but on for instance bacterial products released by the antibiotic carbenicillin treatment.

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