CEFAZOLIN, A NEW SEMISYNTHETIC CEPHALOSPORIN ANTIBIOTIC. IV

ANTIGENICITY OF CEFAZOLIN AND ITS CROSS REACTIVITY WITH BENZYLPENICILLIN, AMPICILLIN AND CEPHALORIDINE

YASUHIRO MINE and MINORU NISHIDA

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.
Osaka, Japan

SACHIKO GOTO and SHOGO KUWAHARA

Department of Microbiology, Toho University, School of Medicine
Tokyo, Japan

(Received for publication January 23, 1970)

The antigenicity of cefazolin and its cross reactivity with benzylpenicillin, ampicillin and cephaloridine were studied. Cefazolin showed a sensitizing activity, as evidenced by the elicitation of specific precipitin antibodies and of hemagglutinating antibodies in experimental animals immunized with protein conjugates of this antibiotic, as do conjugates of penicillins and cephalosporins. Cefazolin gave a minimal cross reactivity, not only with benzylpenicillin but also with ampicillin and cephaloridine. Quantitative hapten inhibition of precipitation, against anti-benzylpenicillin antibodies, by haptens of 7-aminocephalosporanic acid and 6-aminopenicillanic acid was only minimal. This finding suggests the possibility that the cross reaction between cephalosporin derivatives and related penicillins against benzylpenicillin is mediated mainly by the acyl side chain of the molecules.

Cephalosporin antibiotics are substitution products of 7-aminocephalosporanic acid (7-ACA), and differ from the penicillins in that a 6-membered dihydrothiazine ring replaces the 5-membered thiazoline ring of the parent compound. Because of their difference in nuclear structure, there appeared to be little or no cross allergenicity between cephalosporins and penicillins. For this reason, earlier reports indicated that cephalosporins could be safely given to patients known to be hypersensitive to the penicillins.[1,2,3,4]

But recent work has established that the cephalosporins have cross reactivity with benzylpenicillin in animals and, very probably, in man.[5,6,7] Indeed, more recently, a few patients having a history of penicillin allergy were reported to be allergic to cephalosporins.[8,9] Fig. 2 shows possible pathways in the formation of protein conjugates of cephalosporins.[10,11]

Cefazolin (CEZ) is a new cephalosporin C derivative. This substance has a tetrazolylacetyl side chain on the amino group and a 5-methyl-thiadiazolyl-thiomethyl group on the 3-position of the 7-ACA[12]. Because of the structural similarity of penicillins and cephalosporins, it is natural to suppose the existence of some overlaps in allergenicity.

This report describes the antigenicity of cefazolin and its cross reactivity with other cephalosporins and penicillins by means of hemagglutination test, inhibition of hemagglutination test, quantitative hapten inhibition of precipitation test and passive cutaneous anaphylaxis reaction.

Materials and Methods

Antigen

Cefazolin (CEZ, Fujisawa Research Laboratories), potassium benzylpenicillin (PC-G, Fujisawa Pharm. Co., Ltd.), cephaloridine (CER, Glaxo Laboratories), ampicillin (AB-PC, Beecham Research Laboratories), cephalothin (CET, Eli Lilly and Co.). The preparations of phenylacetoamido cephalosporanic acid (FK-1), 2-thienylacetyl-3-2-(5-methyl-1,3,4-thiadiazolyl)-thiomethyl-Δ^3-cephem-4-carboxylate (FK-2), 7-ACA and 6-aminopenicillanic acid (6-APA) were produced in Fujisawa Research Laboratories. Bovine γ-globulin (BGG) used was Fraction II of bovine plasma (Armour Co.). Rabbit albumin crystalline (RSA) was purchased from the Nutritional Biochemicals Corporation.

Preparation of benzylpenicilloyl-protein conjugates

Two hundred mg of PC-G was incubated with 50 mg of RSA or BGG in 5 ml of veronal buffer at 37°C for 24 hours. The pH was maintained by the addition of 1 N NaOH at pH 8.5~11.0. The solution of the resultant conjugate was then dialyzed against buffered saline, pH 8.0 at 4°C for 6 days. The penicilloyl (BPO) content of the conjugates was determined by the penamaldate method[13]. The conjugates contained 32 BPO groups/molecule of RSA and 33 BPO groups/molecule of BGG. Aminobenzylpenicilloyl-protein conjugates were prepared in the same manner and 24 and 28 aminobenzylpenicilloyl groups, respectively, were bound to one molecule of RSA and BGG.

Preparation of cefazolin-protein and cephaloridine-protein conjugates

CEZ or CER was coupled to protein at pH 8.5 to 11.0, following the same procedure described for the conjugates of PC-G.

Experimental animals

White rabbits each weighing 2.0~2.3 kg were used for the production of antisera. Albino strain guinea pigs each weighing 320~350 g were used for passive cutaneous anaphylaxis experiments.

Rabbit immunization schedule

A 4-ml aliquot of antibiotic-RSA conjugate was emulsified with an equal volume of (FREUND's complete) adjuvant; 0.2 ml of this emulsion was given intramuscularly, 0.2 ml intradermally into 2 sites on the abdomen, and 0.6 ml subcutaneously to three white rabbits. The emulsion injections were repeated for 4 or 8 weeks at 4~7 day intervals. One week after the last injection, the rabbits were given as a booster an intramuscular injection of the antibiotic-protein conjugate solution. Bleedings taken on the 5th day after the booster injection were used for antisera. Antisera were collected from three rabbits.

Hemagglutination test

Normal rabbit erythrocytes (RBC) were sensitized with each antibiotic as described by LEY[14]. Four ml of blood from an unimmunized rabbit was mixed with an equal volume of ALSEVERS solution containing 120 mg of the antibiotic. The suspension was incubated for 1 hour at 37°C, centrifuged and washed three times with physiologic saline. The cells were made up to a 2% suspension in physiologic saline. Antisera were prepared from an initial 1:2 dilution and then from a twofold serial dilution in saline. To 0.3 ml of the individual serial dilution of antisera in tray (Tominaga Works Ltd.), 1 drop of the 2% erythrocyte suspension was added. The tray sealed with seal-paper and incubated for 2 hours at 37°C, stored 20 hours at 4°C and the results then read micro-

scopically for hemagglutination, the end points being determined as the highest serum dilution at which clumping of red blood cells occurred.

Inhibition of hemagglutination

Antiserum, 0.5 ml, was added to each 0.5 ml of serial twofold dilutions of inhibitor at an initial concentration of 500 mM/ml, and the dilutions incubated at 37°C for 2 hours. One drop of 2 % sensitized erythrocyte suspension was then added to each 0.3 ml of the mixtures. The remainder of the procedure was the same as that used for the hemagglutination test. Antiserum was used at 4 times the concentration needed to give detectable hemagglutination. Hapten concentrations were expressed as the lowest concentration in millimoles per milliliter which gave complete inhibition of hemagglutination.

Quantitative hapten inhibition of precipitin reaction

To determine the equivalence of antigen and antibody, modified methods described by HEIDELBERGER were used[15]. Antisera and antigens (antibiotic-BGG conjugate) were clarified by centrifugation at 10,000 r.p.m. for 30 minutes. A constant volume (0.5 ml) of antiserum at a 1 : 10 dilution was added to increasing amounts of antigen dissolved in 0.5 ml of 0.15 M phosphate buffer saline, pH 7.8. The mixtures were incubated at 4°C for 48 hours. They were then centrifuged and the precipitates were washed three times with cold phosphate buffer saline and dissolved in 1 N NaOH. The supernatant was analyzed by ring tests in the usual fashion. Protein assay was done by a modified FOLIN-CIOCALTEAU method.[16]

For hapten inhibition studies, 0.4 ml of appropriate dilutions of haptens at an initial concentration of 200 mM/ml were incubated with 0.5 ml of antisera for 20 hours at 4°C. An amount of antigen, at a concentration which might be at equivalence with the above prepared antisera, was added to the mixtures. After 48-hour incubation at 4°C, precipitates were centrifuged at 3,000 r.p.m. for 20 minutes at 4°C and washed three times with the cold phosphate buffer saline. Protein determinations were done by modified FOLIN-CIOCALTEAU method. In order to compare the inhibitory effects among several haptens, the hapten concentration required for 50 % inhibition of precipitation was calculated.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylaxis (PCA) tests were carried out essentially according to the method of OVARY.[17] A group of 5 guinea pigs were used for each antigen. The dorsal skin surface was clipped free of hair and 0.05 ml of the three antisera and saline were injected intradermally at 4 sites on the area. A period of 18 hours was allowed for antibody fixation, 0.5 ml of test antigen (antibiotic-BGG conjugate) mixed with 0.5 ml of 2 % Evans blue was given intravenously into a foot vein. Thirty minutes after antigen challenge, the diameters of the areas of reactions were measured and, at the same time, the dye was extracted by the method of HARADA *et al.*[18] The cross reactivity among three antisera was calculated by the dye contents.

Results

Hemagglutination

The results of hemagglutination tests are summarized in Table 1. Each pooled rabbit serum, immunized with PC-G : RSA, CER : RSA or CEZ : RSA, consisted of 2 lots. Rabbits immunized with CEZ : RSA conjugate produced hemag-

Table 1. Cross reaction of hemagglutination of pooled rabbit antisera immunized with PC-G : RSA, CER : RSA and CEZ : RSA

Antibody	Antigen and serum hemagglutinin titers			
	PC-G sensitized RBC*	CER sensitized RBC	CEZ sensitized RBC	Unsensitized RBC
PC-G : RSA	× 16 × 64	× 8 × 16	× 2 × 8	0 0
CER : RSA	× 4 × 4	× 16 × 32	× 4 × 8	0 0
CEZ : RSA	× 8 × 32	× 4 × 8	× 16 × 64	0 0

glutinating antibodies as did rabbits immunized with PC-G : RSA and CER : RSA. Sera from CEZ : RSA immunized rabbits agglutinated PC-G-sensitized cells in high titer, but agglutinated CER-sensitized cells only to a limited extent. On the other hand, antisera against PC-G : RSA agglutinated CEZ-sensitized cells in low titer.

From these results, it was concluded that cefazolin possesses a sensitizing potential. The results also showed that cefazolin had weak cross reactivity with anti-PC-G and anti-CER sera.

Table 2. Hapten concentrations required for 100 % inhibition of hemagglutination

Antibody	Hapten (mM/ml)*		
	PC-G	CER	CEZ
PC-G : RSA	31.25	62.5	250.0
CER : RSA	0.024	0.012	12.5
CEZ : RSA	50.0	50.0	0.39

* Hapten concentrations are expressed as the lowest concentration in millimoles per milliliter which is required for 100% inhibition of hemagglutination.

However, no quantitative conclusions can be drawn with respect to the cross reactivity, since we could not measure the relative number of antigenic groups on the surface of the coated cells.

Inhibition of Hemagglutination

Table 2 shows inhibition of hemagglutination by PC-G, CER and CEZ haptens. As can be seen, the PC-G and CER hapten concentrations required for 100 % inhibition of hemagglutination, in anti-PC-G sera : PC-G-sensitized cells system, were 31.25 and 62.5 mM/ml, respectively. On the other hand, much higher concentrations of CEZ hapten, such as 250 mM/ml, were required in this system.

In anti-CER sera : CER-sensitized cells system, the PC-G and CER hapten concentrations required were 0.024 and 0.012 mM/ml, respectively, for 100 % inhibition of hemagglutination, but cefazolin required about a 1,000 times higher concentration.

In anti-CEZ sera : CEZ-sensitized cells system, approx. 50 mM/ml of both the PC-G and CER haptens were required for 100 % inhibition. On the other hand, only 0.39 mM/ml of the cefazolin hapten was required for 100 % inhibition.

These results seemed to indicate that cross reactivity between cefazolin and related antibiotics was minimal, but strong cross reaction existed between PC-G and CER.

Quantitative Hapten Inhibition of Precipitin Reaction

The results are shown in Fig. 1 and Table 3. Chemical structures of haptens are listed in Table 4. When the hapten concentrations required for 50 % inhibition were compared, homologous penicillins and cephalosporins were found to be the most effective inhibitors in every antigen-antibody system. For example, in anti-PC-G : PC-G system, PC-G hapten gave 50 % inhibition at 2.5 mM/ml, whereas much higher concentrations of the other haptens were required for 50 % inhibition ; for example a 34-fold greater concentration of cefazolin hapten was required (K rel. 0.029) compared with the PC-G hapten.

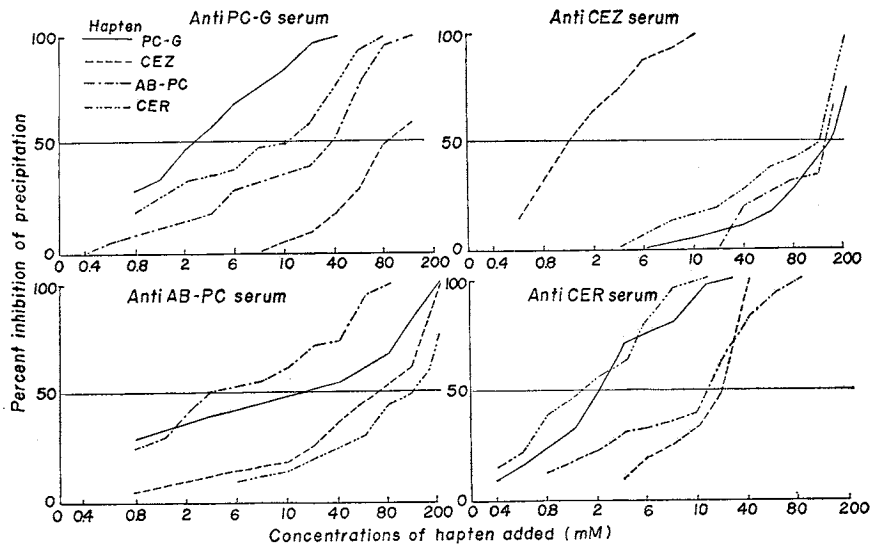
It was also observed that the inhibition by 6-APA and 7-ACA were minimal in this system. Although CER, CET and FK-2 are substitution products of 7-ACA the same acyl side chain, and differ only in the C-3 side chain, they are nearly the same in their inhibitory ability in this system.

Also, PC-G and FK-1 molecules have the same acyl side chain and differ only

Table 3. Hapten concentrations required for 50 % inhibition of quantitative precipitin reaction against PC-G, CEZ, CER and AB-PC antisera

System		Hapten								
		PC-G	CEZ	CER	AB-PC	CET	6-APA	7-ACA	FK-1	FK-2
Anti-PC-G ; PC-G : BGG	$(H)^{mM}_{50\,\%}$	2.5	85.0	10.0	32.0	11.0	>100.0	>100.0	2.7	9.0
	K rel.	1.0	0.029	0.25	0.078	0.227	<0.025	<0.025	0.907	0.278
Anti-CEZ ; CEZ : BGG	$(H)^{mM}_{50\,\%}$	150.0	1.05	100.0	125.0	—	—	—	—	—
	K rel.	0.007	1.0	0.011	0.008	—	—	—	—	—
Anti-CER ; CER : BGG	$(H)^{mM}_{50\,\%}$	1.9	20.0	1.15	14.0	—	—	—	—	—
	K rel.	0.605	0.058	1.0	0.08	—	—	—	—	—
Anti-AB-PC ; AB-PC : BGG	$(H)^{mM}_{50\,\%}$	15.0	64.0	115.0	3.8	—	—	—	—	—
	K rel.	0.253	0.059	0.033	1.0	—	—	—	—	—

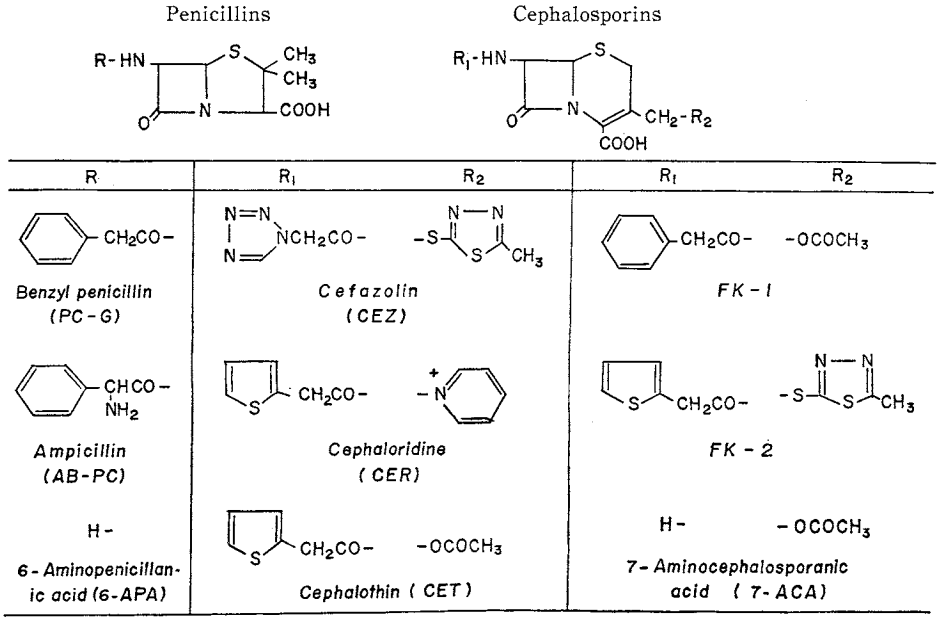
Fig. 1. Hapten inhibition of the precipitation of pooled rabbits antisera against PC-G, CER, AB-PC and CEZ : RSA conjugate by PC-G, CER, AB-PC and CEZ : BGG.



in the structure of the nucleus, 6-APA and 7-ACA, respectively. Nevertheless, 50 % inhibition in this system was observed with PC-G and FK-1 at the same molar concentration.

From these results, it seems likely that the acyl side chain of the penicillins and cephalosporins may play an important part in their cross reactivity with PC-C. In the anti-CER : CER-BGG system, CER and PC-G haptens gave 50 % inhibition at 1.15 and 1.9 mM/ml, respectively, whereas the concentration of cefazolin hapten required for 50 % inhibition was 20.0 mM/ml. Similarly, in anti-AB-PC : AB-PC-BGG system, AB-PC and PC-G haptens gave 50 % inhibition at 3.8 and 15.0 mM/ml,

Table 4. Chemical structures of haptens



respectively. On the other hand, much higher concentrations of CER and CEZ haptens, 115.0 mM/ml and 64.0 mM/ml, respectively, were required. In anti-CEZ : CEZ-BGG system, CEZ hapten was about 95 to 143 times as inhibitory as in other antibiotic haptens.

These results show that cross reactivity between cefazolin and related antibiotics is generally weak.

Passive Cutaneous Anaphylaxis

As shown in Table 5, pooled rabbits antisera gave PCA reactions in all guinea pigs when homologous antibiotic-BGG conjugate was used as the eliciting antigen. Strong cross reactivity was seen with CER : BGG for anti-PC-G sera, whereas cross reactivity between CEZ : BGG and anti-PC-G sera was absent. Similarly, PC-G antigen gave cross reaction at 21.4 % against anti-CER sera. On the other hand, CEZ : BGG antigen elicited weak cross reaction at 9.7 % against anti-CER sera but cross reactions with PC-G : BGG and CER : BGG antigens against anti-CEZ sera were absent.

Table 5. Cross reaction of passive cutaneous anaphylaxis of pooled rabbit antisera against PC-G, CER and CEZ

Antibody		Antigen		
		PC-G : BGG	CER : BGG	CEZ : BGG
Anti-PC-G	Zone (mm)	37.3~33.6	26.2~23.9	—
	Evans (μg)	46	27	—
	Cross rate (%)	100	58.7	0 (?)
Anti-CER	Zone (mm)	31.3~30.6	35.9~34.1	20.9~18.2
	Evans (μg)	22	103	10
	Cross rate (%)	21.4	100	9.7
Anti-CEZ	Zone (mm)	—	—	24.5~21.3
	Evans (μg)	—	—	19
	Cross rate (%)	0 (?)	0 (?)	100

Each value represents the average of five guinea pigs.

Discussion

It is believed that penicillins cause sensitization only if they react with the ε-amino groups of protein to form the stable covalently linked hapten-protein conjugate, and that the most important antigenic determinant in penicillin allergy is the penicilloyl group (Fig. 2).

Investigations into the pathways forming the penicillin antigen have been carried out by the other researchers.[19,20,21] On the other hand, little investigation has been made regarding the cephalosporin antigen.

Fig. 2. Proposed pathways for formation of antigen in penicillin allergy[11].

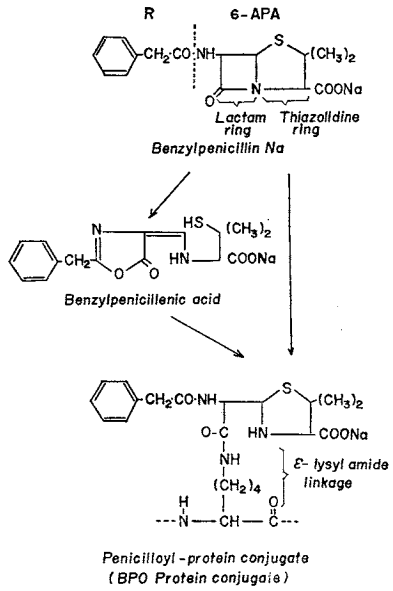
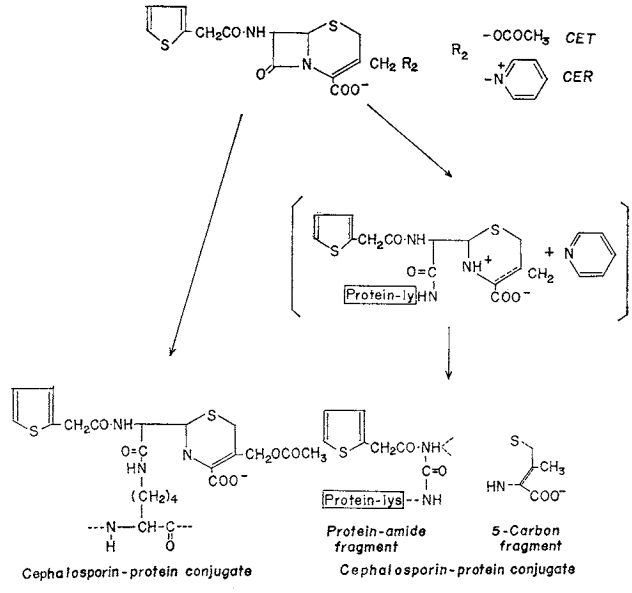


Fig. 3. Proposed pathways for formation of antigen in cephalosporin allergy[10,11].



As shown in Fig. 3, due to the structural similarity of penicillins and cephalosporins, it is supposed that the opening of the β-lactam ring of cephalosporins causes the formation of stable covalently linked conjugates as in penicillins[11]. However, a recent work has shown that cephalosporins lead to rapid fragmentation of the molecule under physiological conditions[10].

In the present study, the antigenicity of cefazolin and its cross reactivity with related antibiotics was studied with antisera against RSA complex, which were incubated with PC-G, AB-PC, CER and CEZ under alkaline conditions. It is postulated from our experiments that cefazolin possesses a sensitizing activity in man as evidenced by the elicitation of specific antibodies in experimental animals.

With regard to the specificity of rabbit antibodies to BPO, LEVINE has already pointed out that they were specifically adapted to a large antigenic unit comprised of the entire BPO group, the lysine side chain and structural configurations of the immunizing carrier proteins by means of PCA, quantitative precipitin and hapten inhibition reactions[22].

From our results in quantitative hapten inhibition of precipitin reaction, cross reactivity of cefazolin antigen against anti-BPO antibodies and anti-CER antibodies was minimal. In contrast, strong cross reaction of CER and CET antigens against anti-BPO antibodies was seen. As was shown earlier by SHIBATA *et al.*, their cross reactivity may be related to the chemical resemblance of thiophene and benzene in the side chain of the compounds[6].

BRANDRISS has reported that CET and BPO antigens against rabbit anti-BPO antibodies and anti-CET antibodies react to nearly the same degree, but CET antigen against anti-6-APA antibodies give weak or negative cross reactions[11]. Our experiment suggested that cross reactions of 6-APA and 7-ACA antigens against anti-BPO antibodies were very weak and that FK-1, which has the same acyl side chain with PC-G, reacted in the same degree with PC-G against anti-BPO antibodies and furthermore CER, CET and FK-2 having the same C-7 side chain, but differing only in the C-3 side chain of 7-ACA, reacted in the same degree against anti-BPO antibodies.

From these results it seems likely that the acyl side chain of penicillins and cephalosporins may play an important part in their cross reactivity with PC-G. In this respect, it is supposed that cefazolin gives only a weak cross reaction against PC-G and CER on account of the considerable difference in the C-7 side chain structure of cefazolin from that of PC-G and CER.

The significance of these results is difficult to assess. Heterogeneity exists in populations of penicillin-induced antibody molecules with respect to the degree of specificity in individual rabbits at different times after immunization (ATSUMI[23]), and to the combining sites on the antibody molecule[24]. Moreover, a number of penicillin antigens have been identified in animals and it is likely that this complex situation also exists in man. While in the present study, whole antisera were used, the use of purified globulin fractions would help to determine the significance of results obtained.

A further consideration results from the suggestion that a macromolecular protein or peptide residue in penicillins and cephalosporins is responsible for some types of allergic reactions and this residue may be carried over from the biosynthetic process or may form, in solution, along with another polymer by degradation of the primary ring structure.[25,26,27]

These facts make the understanding of the analysis of penicillin allergy difficult. It is possible however to reduce the risk of hypersensitivity by modifications of acyl side chain.

Although we report that cefazolin has minimal cross reactivity with benzylpenicillin, cefazolin should be given with care to patients with known hypersensitivity to penicillins.

Acknowledgement

The authors wish to thank Dr. Y. HORIUCHI, Hokkaido University School of Medicine and Dr. H. NAKANO, Director of Fujisawa Research Laboratories and Dr. S. KUMADA for guidance and encouragement.

Bibliography

1) WALTERS, E. W.; M. J. ROMANSKY & A. C. JOHNSON: Cephalothin-Laboratory and clinical studies in 109 patients. Antimicr. Agents & Chemoth. -1963 : 247~253, 1964
2) WEINSTEIN, L.; K. KAPLAN & T. W. CHANG: Treatment of infection in man with cephalothin. J. Amer. Med. Ass. 189 : 829~833, 1964
3) PHILSON, J. R.; C. F. CLANCY & J. D. ALEXANDER: Cephalothin therapy in bacterial infections. Antimicr. Agents & Chemoth. -1963 : 267~271, 1964
4) GRIFFITH, R. S. & H. R. BLACK: Cephalothin, a new antibiotic; Preliminary clinical and laboratory studies. J. Amer. Med. Ass. 189 : 823~828, 1964
5) HARVEY, R. G. & H. M. MARY: Immune cross-reactivity of penicillin and cephalothin. Nature 216 : 1026~1027, 1967
6) SHIBATA, K.; T. ATSUMI, Y. HORIUCHI & K. MASHIMO: Immunological cross-reactivities of cephalothin and its related compounds with benzylpenicillin. Nature 212 : 419~420, 1966
7) BATCHELOR, F. R.; M. D. JANET, R. D. WESTON & A. W. WHEELER: The immunogenicity of cephalosporin derivatives and their cross-reaction with penicillin. Immunology 10 : 21~33, 1966
8) THOBURN, R.; J. E. JOHNSON & L. E. CLUFF: Studies on the epidemiology of adverse drug reactions. J. Amer. Med. Ass. 198 : 345~348, 1966
9) GRIECO, M. H.: Cross-allergenicity of the penicillins and the cephalosporins. Arch. Intern. Med. 119 : 141~146, 1967

10) NEWTON, G.G.F. & J.M.T. HAMILTON-MILLER: Cephaloridine; Chemical and biochemical aspects. Postgrad. Med. J. 43 : 10~13, 1967
11) BRANDRISS, M. W.; J. W. SMITH & H. G. STEIMAN: Common antigenic determinants of penicillin G, cephalothin and 6-aminopenicillanic acid in rabbit. J. Immunol. 94 : 696~704, 1965
12) KARIYONE, K.; H. HARADA, M. KURITA & T. TAKANO: Cefazolin, a new semisynthetic cephalosporin antibiotic. I. Synthesis and chemical properties of cefazolin. J. Antibiotics 23 : 131~136, 1970
13) LEVINE, B. B.: N-(α-D-Penicilloyl) amines as univalent hapten inhibitors of antibody-dependent allergic reactions to penicillin. J. Med. Pharm. Chem. 5 : 1025~1034, 1962
14) LEY, A. B.; J. P. HARRIS, M. BRINKLEY, B. LILES & J. A. JACK: Circulating antibody directed against penicillin. Science 127 : 1118~1119, 1958
15) KABAT, E. A. & M. M. MAYER: Experimental immunochemistry. 2nd Ed. pp. 22~96 (Chap. 2) Charles C. Thomas, Springfield, Illinois, U.S.A. 1961
16) FOLIN, O. & V. CIOCALTEU: Tyrosine and tryptophan determination in proteins. J. Biol. Chem. 73 : 627~650, 1927
17) OVARY, Z.: Progress in allergy. 5 : 459, 1958 (S. Karger, Basel & New York)
18) HARADA, M.; M. TAKEUCHI & K. KATAGIRI: Study on quantitative determination of passive cutaneous anaphylaxis and effect of antihistamines on anaphylaxis in guinea pigs. Allergy (Japan) 15 : 1~7, 1966
19) LEVINE, B. B.: Immunochemical mechanism involved in penicillin hypersensitivity in experimental animals and in human beings. Fed. Proc. 24 : 45~50, 1965
20) BATCHELOR, F. R.; J. M. DEWDNEY & D. GAZZARD: Penicillin allergy; The formation of the penicilloyl determinant. Nature 206 : 362~364, 1965
21) PARKER, C. W.: Immunochemical mechanism in penicillin allergy. Fed. Proc. 24 : 51~54, 1965
22) LEVINE, B. B.: Studies on the dimensions of the rabbit antibenzylpenicilloyl antibody-combining sites. J. Exp. Med. 117 : 161~183, 1963
23) ATSUMI, T.; K. NISHIDA, Y. KINOSHITA, K. SHIBATA & Y. HORIUCHI: The heterogenity of combining sites of anti-benzylpenicilloyl antibodies obtained from individual rabbits; Fractionation of antibodies with a specific immunoabsorbent. J. Immunol. 99 : 1286~1293, 1967
24) ATSUMI, T.; M. ADACHI, Y. KINOSHITA, M. KAWASAKI & Y. HORIUCHI: The heterogeneity of combining sites of anti-benzylpenicilloyl antibodies obtained from individual rabbits; Changes in combining sites of γG and γM antibodies during the immune response. J. Immunol. 101 : 1016~1022, 1968
25) WESTON, R. D.: Penicilloylated protein contaminating 6-aminopenicillanic acid and benzylpenicillin. Antimicr. Agents & Chemoth. -1967 : 553~559, 1968
26) STEWART, G. T.: Macromolecular residue contributing to the allergenicity of penicillins and cephalosporins. Antimicr. Agents & Chemoth. -1967 : 543~549, 1968
27) BATCHELOR, F. R.; J. M. DEWDNEY, J. G. FEINBERG & R. D. WESTON: A penicilloylated protein impurity as a source of allergy to bezylpenicillin and 6-aminopenicillanic acid. Lancet 1967-1 : 1175~1188, 1967