IMMUNOLOGICAL CROSS-REACTIVITIES OF SULFOCEPHALOSPORINS

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The immunological cross-reaction among sulfocephalosporins (cefsulodin, SFC-I, SFC-II), cephaloridine, sulbenicillin, penicillin G and 7-aminocephalosporanic acid has been investigated. A specific antibody was produced in rabbits against several structurally related haptens. The sensitization was conducted with a hapten-human serum albumin conjugate, which was prepared in an alkaline solution, with FREUND's complete adjuvant. A cross-reaction was clearly demonstrated between sulfocephalosporins and sulbenicillin, but not between sulfocephalosporins and cephaloridine, penicillin G or 7-aminocephalosporanic acid.

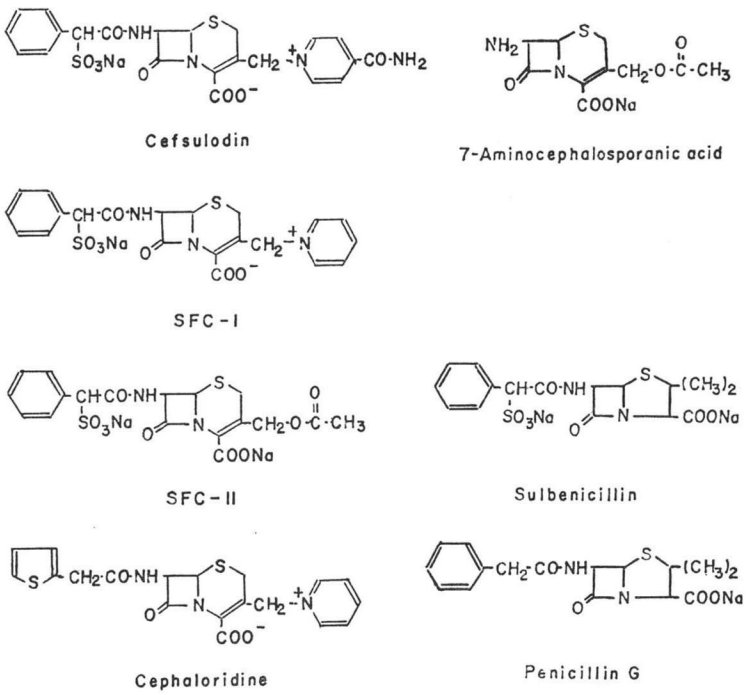
The chemical structures of cephalosporins differ from the penicillins by having the cephem ring in place of the penam ring. Earlier reports indicated that cephalosporin derivatives can be given to patients who are sensitive to penicillin G (PCG)[1~5]. Later, a few cases of allergic reactions on the first exposure to cephalothin (CET) have been reported in the patients sensitive to PCG[6~11]. BATCHELOR *et al.*[12] and SHIBATA *et al.*[13] reported that the immunological cross-reactivity between CET and PCG may be related to the chemical resemblance between the 2-thienylacetic acid at the 7-position of CET and the phenylacetic acid at the 6-position of PCG. Many cephalosporins have a variety of side chain acyl groups at the 7-position that are different from those on the 6-position of penicillins, and this probably would account for relatively rare cases of immunological cross-reactions between these groups of β-lactam antibiotics[14~18].

Penicillins have two immunologically active sites; one is an acyl side chain at the 6-position which is related to a specific reactivity and the other is the five-membered thiazolidine which is related to the cross-reactivity among a wide variety of penicillin derivatives[19,20]. However, the immunological reactivities of a six-membered dihydrothiazine ring and the side chain at the 3-position in cephalosporins are not clear.

Cefsulodin [3-(4-carbamoyl-1-pyridiniomethyl)-7β-(D-α-sulfophenylacetamido)-ceph-3-em-4-carboxylate monosodium salt, previously named SCE-129], a new antipseudomonal cephalosporin, has sulfophenylacetic acid at the 7-position and carbamoyl-pyridinomethyl at the 3-position in its structure[21~24]. It was suggested, therefore, that cefsulodin would have a cross-reactivity with sulbenicillin (SBPC) which has the same side chain at the 6-position of the penicillin (Fig. 1).

The present study was designed to characterize the immunological cross reactivity of cefsulodin with related compounds, and the immunological extent of a six-membered dihydrothiazine ring and the side chain at the 3-position by using various immunological techniques and several reference compounds such as SFC-I (7-sulfophenylacetic acid; 3-pyridiniomethyl), SFC-II (7-sulfophenylacetic acid; 3-acetoxymethyl), cephaloridine (CER) (7-thienylacetic acid; 3-pyridiniomethyl), sulbenicillin (SBPC) (6-sulfophenylacetic acid), penicillin G (PCG) (6-phenylacetic acid) and 7-aminocephalosporanic acid (7 ACA) (3-acetoxymethyl).

Fig. 1. Chemical structures of cephalosporins and penicillins.



Materials and Methods

Chemicals

Cefsulodin, SFC-I and SFC-II were prepared in Takeda Chemical Industries, Ltd., Osaka, Japan. Sulbenicillin (SBPC) and penicillin G (PCG) were the commercial products of Takeda Chemical Industries, Ltd. Cephaloridine (CER) was purchased from Shionogi Pharmaceutical Co. 7-Aminocephalosporanic acid (7 ACA) was supplied by Ciba-Geigy Pharmaceutical Co. Human serum albumin (HSA) and bovine gamma globulin (BGG) were purchased from Nutritional Biochemical Co.

Preparation of Hapten-Protein Conjugate

Six hundred mg of hapten and 100 mg of HSA or BGG were dissolved in 10 ml of physiological saline. The pH was adjusted to 10~11 with 1 N NaOH, and the solution was incubated at 37°C for 24 hours. The resultant conjugate was dialyzed against a large volume of physiological saline at pH 8 for 72 hours, and then through a column (3 × 100 cm) of Sephadex G-25 (fine) for removing the unreacted hapten. The final concentration of hapten-protein conjugate was adjusted to 10 mg protein per ml. The epitope density of penicillin-protein conjugates was assayed by the PENAMALDATE method[25], and the number of penicilloyl groups per mol protein were found to be larger than 30. The epitope density of cephalosporin-protein conjugates assayed by the amino acid analysis method[26] was more than 20 cephalosporin moieties per mol protein.

Antisera

Rabbits antisera for the antigens were prepared in hybrid rabbits weighing 2.5~3 kg by injecting an emulsion of hapten-HSA conjugate in an equal amount of FREUND's complete adjuvant (Difco) at multiple sites. Initial injection was given at 20 sites intradermally on the abdomen, using 0.1 ml each and 2 ml in total. After one week, 2 ml of the emulsified mixture was injected intramuscularly. Thereafter injections were repeated at weekly intervals by the intradermal and intramuscular routes. One week after the fourth injection, the rabbits were administered intramuscularly 120 mg of hapten alone as a booster. Five to ten days after the last injection of the hapten, the sera were obtained. Three sera showing positive precipitin reaction against respective hapten-BGG conjugate were pooled and stored at −20°C until use.

Quantitative Precipitin Reaction

The studies were done according to the procedure of KABAT and MAYER[27]. Hapten-BGG conjugate and antisera were diluted with 1/15 M phosphate buffer in saline (pH 7.4) (PBS). To a series of different concentrations of the hapten-BGG solution (0.5 ml each) was added 0.5 ml each of an antiserum. After 2 hours of incubation at 37°C, the mixtures were stored at 4°C for 48 hours. The precipitates were washed 3 times with cold PBS, and the protein content of the precipitates was measured by LOWRY's method[28]. The amount of protein was read and expressed in BGG equivalents

from the standard curve.

Quantitative Hapten-Inhibition of Precipitin Reaction

The method of PAULING *et al.*[29] was used. A series of increasing amounts of a hapten dissolved in 0.5 ml of PBS were added to 0.5 ml aliquots of an antiserum. After 2-hour incubation at 37°C, the corresponding hapten-BGG conjugate was added to the hapten-antiserum mixture at the dose which produced a maximum of homologous precipitin reaction of hapten-BGG conjugate dissolved in 1 ml of PBS. After an additional 2-hour incubation at 37°C and subsequent 48-hour storage at 4°C, the protein content of the precipitates was measured by the method described above. The inhibitory effect of hapten was calculated by comparing the amount of protein with that in the precipitate of the tubes with and without hapten.

Immunodiffusion Analysis

This assay was performed by the method developed by OUCHTERLONY[30]. Agar plates containing 0.8% agar (Special agar B, Wako Pure Chemical Industries, Ltd.), 0.85% NaCl and 0.01% NaN_3 were used, and the distance between the wells for antigen and antiserum was set at 5 mm.

Passive Cutaneous Anaphylaxis (PCA) Reaction

Determination of PCA reacting antibodies was performed in guinea pigs according to the method of OVARY[31]. One-tenth ml of antisera diluted in physiological saline was injected intradermally, and a mixture of hapten-BGG conjugate and 1% Evans blue solution was challenged by the intravenous route 6 hours after sensitization. At 30 minutes after challenge, the diameters of dye leakage were measured from two distinct directions. The results were expressed as the average diameter of dye leakage.

Results

Quantitative Precipitin Test

The results of the quantitative precipitin analysis of rabbit anti-hapten-HSA sera by homologous and heterologous hapten-BGG conjugates are shown in Table 1. Three anti-sulfocephalosporin-HSA sera and anti-SBPC-HSA serum reacted strongly against three sulfocephalosporin-BGG and SBPC-BGG conjugates, and the percentages of the precipitable antibody-antigen complex based on the total amount precipitated against homologous antigen at a maximum reaction were found to be almost the same among these three sulfocephalosporins. The anti-sulfocephalosporin-HSA sera also reacted strongly with SBPC-BGG conjugate, whereas anti-sulfocephalosporin-HSA sera reacted only slightly with PCG-, CER- and 7 ACA-BGG conjugates. The reaction of anti-SBPC-HSA serum was

Table 1. Cross reactions between sulfocephalosporins and related antibiotics measured by quantitative precipitation.

Hapten-BGG	Anti-hapten-HSA serum					
	Cefsulodin	SFC-I	SFC-II	Sulbenicillin	Penicillin G	Cephaloridine
Cefsulodin	100*	119.3	82.1	69.4	4.9	21.8
SFC-I	100.5	100	76.0	57.0	4.9	19.9
SFC-II	113.1	113.9	100	50.4	2.4	19.4
Sulbenicillin	115.1	112.3	111.8	100	38.8	17.1
Penicillin G	14.1	7.0	5.7	28.7	100	70.1
Cephaloridine	6.8	14.0	6.2	10.7	52.1	100
7 Aminocephalosporanic acid	8.3	17.5	9.3	9.9	4.9	25.5

* Relative value of precipitable antibody and antigen complex at point of maximum precipitation, taking the absolute rate of homologous system as 100.

moderate with three sulfocephalosporin-BGG conjugates, and weak with PCG-BGG conjugate, but apparently it did not react with CER- or 7 ACA-BGG conjugates. Anti-PCG-HSA serum reacted strongly with homologous antigen. The reaction between anti-PCG-HSA serum and CER-BGG conjugate was stronger than that between anti-PCG-HSA serum and SBPC-BGG conjugate. Anti-PCG-HSA serum did not show any significant reactivity against three sulfocephalosporin- and 7 ACA-BGG conjugates. Anti-CER-HSA serum reacted with CER- and PCG-BGG conjugates strongly, and the homologous antiserum-antigen reaction was stronger than the reaction observed between anti-CER-HSA serum and PCG-BGG conjugates. Anti-CER-HSA serum did not react significantly with the other hapten-BGG conjugates.

Quantitative Hapten Inhibition Test

The ability of haptens to inhibit the precipitin reaction occurring between an anti-hapten-HSA serum and the corresponding hapten-BGG conjugate at an optimum ratio was compared (Table 2). Each hapten was used at concentrations 0.032~100 mM. The precipitin reactions between three anti-sulfocephalosporin-HSA sera and three sulfocephalosporin-BGG conjugates were inhibited strongly with sulfocephalosporins and sulbenicillin, but were not inhibited even with 100 mM of PCG, CER or 7 ACA. The precipitin reaction between anti-SBPC-HSA serum and SBPC-BGG conjugate was inhibited strongly with SBPC, and a moderate inhibition was observed with sulfocephalosporins. While the precipitin reaction between anti-SBPC-HSA serum with SBPC-BGG conjugate was not inhibited with 100 mM of PCG, CER and 7 ACA. The precipitin reaction between anti-PCG-HSA serum and PCG-BGG conjugate was inhibited at almost the same concentration with PCG and CER, but was not inhibited by 100 mM of either sulfocephalosporins, SBPC or 7 ACA. The precipitin reaction between anti-CER-HSA serum with CER-BGG conjugate was inhibited at almost the same concentration with CER and PCG, but was not inhibited with 100 mM of either sulfocephalosporins, SBPC or 7 ACA.

Table 2. Hapten concentration (mM) required for 50% inhibition of quantitative precipitation of hapten-BGG with anti-hapten-HSA serum.

Hapten	System					
	Cefsulodin	SFC-I	SFC-II	Sulbenicillin	Penicillin G	Cephaloridine
Cefsulodin	0.29	1.2	0.39	10.6	70.0	>100
SFC-I	0.50	0.73	0.55	5.1	34.0	>100
SFC-II	0.59	0.56	1.03	4.4	46.0	>100
Sulbenicillin	0.63	0.91	0.8	0.7	>100	>100
Penicillin G	>100	>100	>100	>100	1.12	0.51
Cephaloridine	>100	>100	>100	>100	2.5	0.52
7 Aminocephalosporanic acid	>100	>100	>100	>100	>100	>100

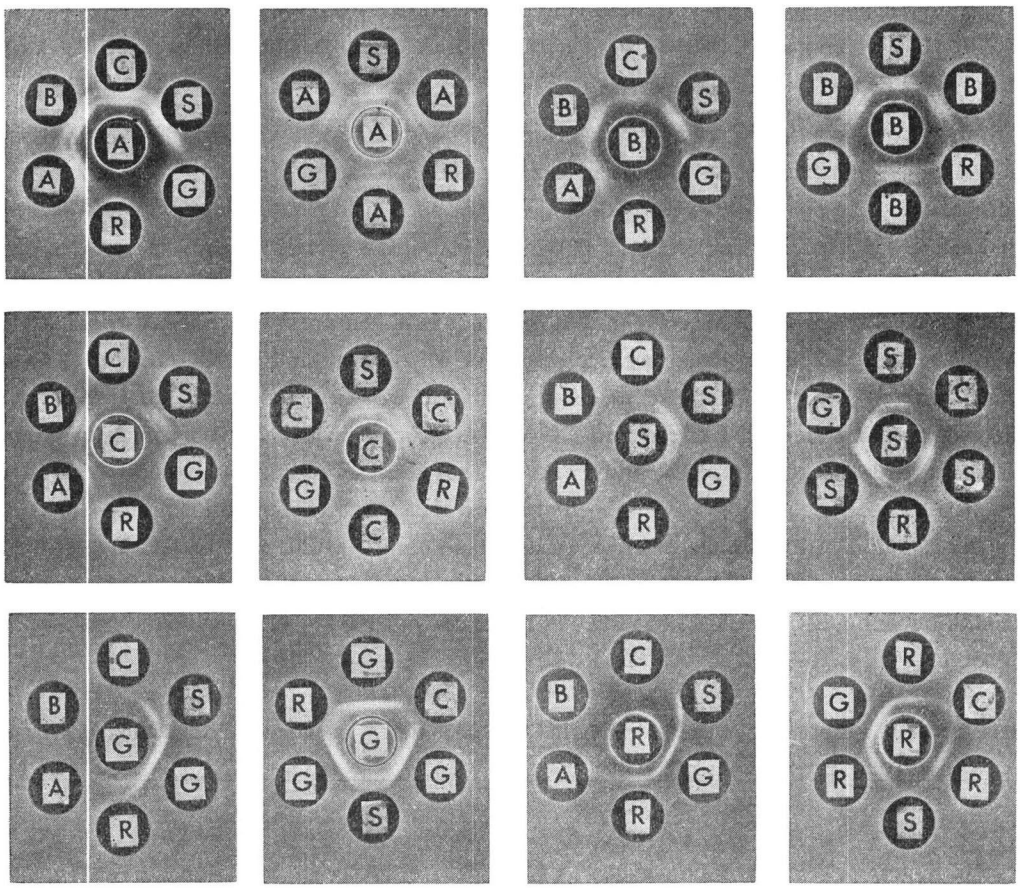
Immunodiffusion Test

All antisera to be tested were filled into the center hole. As shown in Fig. 2, two distinct precipitin bands developed between each anti-sulfocephalosporin-HSA serum and sulfocephalosporin- or SBPC-BGG conjugates. These precipitin bands fused into each other. No precipitin band was obtained between each anti-sulfocephalosporin-HSA serum and PCG- or CER-BGG conjugates.

Fig. 2. Agar precipitation of anti-hapten-HSA serum with hapten-BGG.

Center hole
- A: Anti-cefsulodin-HSA serum
- B: Anti-SFC-I-HSA serum
- C: Anti-SFC-II-HSA serum
- S: Anti-SBPC-HSA serum
- G: Anti-PCG-HSA serum
- R: Anti-CER-HSA serum

Circumference hole
- A: Cefsulodin-BGG (1 : 20)
- B: SFC-I-BGG (1 : 20)
- C: SFC-II-BGG (1 : 20)
- S: SBPC-BGG (1 : 20)
- G: PCG-BGG (1 : 20)
- R: CER-BGG (1 : 20)



Anti-SBPC-HSA serum formed two precipitin bands with SBPC-BGG conjugate, and one precipitin band with each sulfocephalosporin-BGG conjugates. The precipitin bands formed between anti-SBPC-HSA serum and each sulfocephalosporin-BGG conjugate were fused into each other. Between anti-SBPC-HSA serum and PCG-BGG conjugate one precipitin band was formed and fused into the inner precipitin band between anti-SBPC-HSA serum and SBPC-BGG conjugate. No precipitin band was observed between anti-SBPC-HSA serum and CER-BGG conjugate. Anti-PCG-HSA serum and PCG-BGG conjugate formed two precipitin bands and the outer precipitin band was fused into the precipitin bands between anti-PCG-HSA serum and SBPC- or CER-BGG conjugates. Anti-CER-HSA serum and CER- or PCG-BGG conjugate formed one precipitin band and these precipitin bands fused into each other. No precipitin band was observed between anti-CER-HSA serum and sulfocephalosporin- or SBPC-BGG conjugates.

Passive Cutaneous Anaphylaxis Test

In the passive cutaneous anaphylaxis (PCA) test in the skin of guinea pigs, an optimum amount of each anti-hapten-HSA serum and the corresponding hapten-BGG conjugate which showed almost the same reaction was examined. The optimum doses of anti-hapten-HSA sera and hapten-BGG conjugates are shown in Table 3. At the optimum doses these anti-sulfocephalosporin-HSA sera and anti-SBPC-HSA serum reacted strongly with sulfocephalosporin-BGG conjugates and SBPC-BGG conjugate. The anti-sulfocephalosporin-HSA sera did not react with PCG- and CER-BGG conjugates. Anti-PCG- and anti-CER-HSA sera usually reacted with PCG- and CER-HSA sera, but did not react with three sulfocephalosporin-BGG conjugates. Anti-PCG-HSA serum reacted with SBPC-BGG conjugate.

Table 3. Cross reactivity of passive cutaneous anaphylaxis (PCA) reaction of hapten-BGG with anti-hapten-HSA serum.

Hapten-BGG		Anti-hapten-HSA serum					
		Cefsulodin (1:100)	SFC-I (1:100)	SFC-II (1:20)	Sulbenicillin (1:100)	Penicillin G (1:100)	Cephaloridine (1:50)
Cefsulodin	(1:10)	14.0*	14.7	14.2	12.7	0	0
SFC-I	(1:10)	14.0	13.2	13.3	13.5	0	0
SFC-II	(1:20)	13.7	13.5	14.3	13.5	0	0
Sulbenicillin	(1:20)	12.5	12.3	12.7	13.5	10.3	0
Penicillin G	(1:20)	0	0	0	12.0	14.3	12.3
Cephaloridine	(1:10)	0	0	0	0	10.5	14.3

* Each value represents the average diameter (mm) of dye leakage at the antiserum injection sites.

Discussion

Specific antibodies were produced in the rabbits sensitized by a mixture of sulfocephalosporin-HSA conjugates and FREUND's complete adjuvant. Haptens were conjugated with a carrier protein by a covalent bond in an alkaline solution. Anti-sulfocephalosporin-HSA sera reacted with sulfocephalosporin- and SBPC-BGG conjugates. The structures of cephalosporins contain a common skeleton of a six-membered dihydrothiazine ring, thus differ from those of penicillin in the functional groups at the 3-position, *e.g.*, SFC-I and CER have a pyridiniomethyl group and SFC-II and 7 ACA have an acetoxymethyl group. Sulfocephalosporins did not show a cross-reaction with CER and 7 ACA. These results suggest that the antigenic determinant of sulfocephalosporins is the acyl side chain at the 7-position. Although penicillins appear to be able to react with protein in several ways, it may be stated that an important haptenic determinant is the penicilloyl-protein conjugate, however, the mechanism of binding of cephalosporin with protein is not clear. ASSEM and VICKERS[15] reported that an important haptenic determinant of hemagglutinin in the cephalosporin-sensitive patients is cephalosporoyl-protein conjugate. On the other hand, NEWTON and HAMILTON-MILLER[32] suggested that when the β-lactam of a cephalosporin is opened, the cephalosporins would undergo extensive fragmentation, and the haptenic determinants remaining after conjugation of cephalosporins and proteins would be different in structure from that of penicillins. In our present studies the following results were made clear: (1) No cross-reaction was observed among sulfocephalosporins (cefsulodin, SFC-I, SFC-II), CER and 7 ACA. These compounds share cephem ring as a common skeleton. SFC-I and CER have the pyridiniomethyl side chain, and SFC-II and 7 ACA have the acetoxymethyl group at the 3-position of the cephem ring. (2) Cefsulodin, SFC-I, SFC-II and SBPC inhibited the reaction between anti-sulfocephalosporin-HSA sera and sulfocephalosporin-BGG conjugates, and the inhibitory activities of cefsulodin, SFC-I and SFC-II against the reaction between anti-SBPC-HSA

serum and SBPC-BGG conjugate were lower than that of sulbenicillin. As previously reported[20], SBPC has two antigenic active sites ascribable to the acyl side chain at the 6-position and the five-membered thiazolidine ring. These findings suggest that cephalosporins cannot form compounds analogous to the penicilloyl-protein conjugates, and the six-membered dihydrothiazine ring and the acyl side chain at the 3-position may not show antigenic activities.

On the other hand, it has been reported that the specific antibody was produced after the sensitization by the mixture of penicillins or cephalosporins with FREUND's complete adjuvant. Further studies are required to elucidate the antigenic specificity of the cephem ring and the side chain at the 3-position of these antibiotics. Although the cross antigenicity as demonstrated in experiments described here does not necessarily mean cross allergenicity in patients, the facts that PCG-sensitive patients have the high reactivity against CET[6~11], and that the cross-reactivities were observed between PCG and CET[12,13] and SBPC and cefsulodin *in vitro* suggest that sufficient care must be exercised when cefsulodin is used in the SBPC-sensitive patients.

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