DIFFERENCES IN IMMUNE RESPONSES TO ANTIBIOTICS IN THREE GUINEA-PIG STRAINS

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ABSTRACT — The present experiments were undertaken to clarify the differences in humoral immune response to two β -lactam antibiotics, benzylpenicillin potassium (PcG) and cephalothin sodium (CET), in guinea-pig strains. Guinea pigs of three different strains, Hartley, Strain 2 and Strain 13, were immunized subcutaneously with PcG (10 mg/body) 1 or 3 times a week, 10 times in total, or with CET (20 mg/body) 3 times a week, 12 times in total. Humoral immune responses to the two antibiotics were assessed by passive cutaneous anaphylaxis (PCA) and active systemic anaphylaxis (ASA) reactions. The relative intensities of the responses to PcG and CET detected by PCA reaction were Hartley > Strain 2 > Strain 13. On the other hand, Hartley and Strain 13 guinea pigs displayed high responses to the two antibiotics by ASA reaction, and Strain 2 exhibited a relatively low response. Based on these results, it was clarified that the Hartley strain, which is the most common strain used in ordinary allergenicity tests, showed the highest response to the two antibiotics tested in PCA and ASA reactions.

KEY WORDS : Benzylpenicillin potassium, Cephalothin sodium, Immune response, Hartley, Strain 2, Strain 13

INTRODUCTION

Passive cutaneous anaphylaxis (PCA) and active systemic anaphylaxis (ASA) reactions with low-molecular drugs and chemicals in guinea pigs have been reported for predicting drug allergic reactions in pre-clinical safety assessments (Nagatahira *et al.*, 1989; Yoshida *et al.*, 1989; Katsutani and Shionoya, 1992; Shinkai *et al.*, 1992; Verdier *et al.*, 1994; Nagami *et al.*, 1995). A comparison was made among different strains of guinea pig with respect to the humoral immune responses to low-molecular compounds. Previous studies have been carried out generally using high-molecular antigens (Schlossman *et al.*, 1965; McDevitt and Benacerraf, 1969; Haslov *et al.*, 1990). Furthermore, such studies have been performed mostly in mice (Tanioka and Esaki,

1968; Takeuchi and Harada, 1985; Takeuchi and Harada, 1987; Kimura *et al.*, 1991), and are only performed rarely in guinea pigs (Polak *et al.*, 1968; Maurer *et al.*, 1984). Our previous results indicated that different immune responses were evoked in three guinea-pig strains immunized with sodium 2,4,6-trinitrobenzenesulfonate dihydrate (TNBS) without adjuvant (Aida *et al.*, 1997). Since drug allergies to β -lactam antibiotics are known to occur clinically (Levine and Ovary, 1961; Siegel and Levine, 1964; Levine and Redmond, 1969; Ahlstedt and Kristofferson, 1982; Erffmeyer, 1986; Weiss and Adkinson, 1988; Haare and Baldo, 1990; Park and Kitteringham, 1990), allergenicity evaluations on the antibiotics by PCA and ASA reactions are considered to be important. The present experiments were undertaken to elucidate the differences in the humoral immune responses to two β -lactam antibiotics, benzylpenicillin potassium (PcG) and cephalothin sodium (CET),

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in three strains of guinea pig (Hartley, Strain 2 and Strain 13) possessing different major histocompatibility complexes (Chiba and Sugimoto, 1983) by means of PCA and ASA reactions.

MATERIALS AND METHODS

Animals

Outbred Hartley (female, 7 weeks old) and inbred Strain 2 (female, 5~10 weeks old) and Strain 13 (male and female, 7~17 weeks old) guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were individually housed in metal net cages (Shin Toyo Industries, Kawaguchi, Japan) placed in a room where temperature, lighting and ventilation were set at $24 \pm 3^\circ\text{C}$, 14 hr (6:00-20:00)/day with a luminous intensity of about 200 luxes, and about 10 times/hr, respectively. They had free access to solid food RC4 (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. They were acclimatized for about 3 weeks before immunization.

After the Hartley guinea pigs (female, 7 weeks old) were acclimatized to the above conditions for about 9 weeks, 12 animals, each weighing 576-725 g, were provided for the homologous PCA reaction.

Reagents

PcG (Meiji Seika Kaisha, Ltd., Tokyo, Japan) and CET (Shionogi & Co., Ltd., Osaka, Japan) were used as immunogens in the present experiments. Evans' blue was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), physiological saline from Otsuka Pharmaceutical Industries (Tokushima, Japan) and bovine serum albumin (BSA) from Sigma Chemical Company (St. Louis, MO).

The reaction products of PcG and BSA, or CET and BSA were prepared according to the following conventional method (Kimura *et al.*, 1990). PcG (500 mg) and BSA (200 mg) were dissolved in 10 ml of 0.05 M triethylamine buffer (pH 10.5). The solution was maintained at pH 11.0 by adjusting with 2 N NaOH at 37°C for 24 hr. After that, the solution was dialyzed in water at 4°C for 4 days, supplemented with NaCl at a concentration of 0.9%, and then filtered through a $0.45 \mu\text{m}$ membrane filter (SM16555, Sartorius GmbH, Germany). The

protein content was 12 mg/ml as assayed according to the Folin method (Lowry *et al.*, 1951). The conjugation ratio (moles hapten per mole protein) was 20.1 as assayed according to the Penamaldade method (Levine, 1962).

CET (1 g) and BSA (200 mg) were dissolved in 20 ml of 0.1 M carbonate buffer (pH 10.0). The solution was maintained at pH 10.0 by adjusting with 2 N NaOH at 37°C for 24 hr. After that, the solution was dialyzed in water at 4°C for 4 days, supplemented with NaCl at a concentration of 0.9%, and then filtered through a $0.45 \mu\text{m}$ membrane filter. The protein content was 7 mg/ml as assayed according to the Folin method (Lowry *et al.*, 1951). The conjugation ratio was 36.9 as assayed according to the conventional method (Kimura *et al.*, 1990).

Preparation of test samples

PcG or CET was dissolved in physiological saline to make a 10% solution, and the volumes of the administration dose for PcG and CET were fixed at 0.1 ml/body and 0.2 ml/body, respectively.

Experimental protocol

Subcutaneous injection was selected as the route of administration of PcG and CET. Based on the results of a preliminary experiment, 10 mg/body and 20 mg/body were employed as the administration doses of PcG and CET, respectively. Samples were administered subcutaneously to different sites under the dorsal skin each administration day. PcG sample was administered for 4 weeks, once the 1st week, and 3 times the 2nd~4th weeks, 10 times in total. CET sample was administered for 4 weeks, 3 times a week, 12 times in total. Blood was sampled on the 40th day, and serum samples were used for PCA reaction. ASA reaction was performed on the 58th days.

Grouping

Three groups for PcG were used in the present study, with the 1st treatment group consisting of 6 Hartley guinea pigs, the 2nd treatment group consisting of 6 Strain 2 guinea pigs, and the 3rd treatment group consisting of 4 Strain 13 guinea pigs. In addition, three groups for CET were used, with the 4th treatment group consist-

ing of 6 Hartley guinea pigs, the 5th treatment group consisting of 6 Strain 2 guinea pigs, and the 6th treatment group consisting of 5 Strain 13 guinea pigs.

PCA reaction

The PCA reaction was performed as described by Ovary (1964). Sera obtained from groups 1-6 were diluted 5, 10, 20, 40, 80 and 160 times. After that, 0.1 ml of these diluted serum samples was intracutaneously injected into the shaved backs of 2 recipient Hartley guinea pigs. A mixture of an eliciting antigen (0.5 ml) and 2% Evans' blue solution (0.5 ml) was then injected into the ear vein 24 hr after the intracutaneous injection; a 4 mg/ml solution of PcG-BSA or CET-BSA was used as the eliciting antigen. At 30 min to 1 hr after the challenge, the sites injected with the diluted serum samples were observed. The PCA titer was expressed as the reciprocal of the highest dilution of a test serum that gave a positive reaction of cutaneous anaphylaxis.

ASA reaction

As eliciting antigens for ASA, PcG-BSA and CET-BSA were used in groups 1-3 and groups 4-6, respectively; a 4 mg/ml solution (0.5 ml) of each eliciting antigen was injected into the ear vein of the guinea pigs. The animals were observed for 1 hr and the intensity of anaphylactic reaction was rated as described below.

0 : Normal

1 : Cyanosis, cough symptoms, defecation

2 : Intense cyanosis, suffocative symptoms, recumbency, convulsion

3 : Death by shock

RESULTS AND DISCUSSION

The immune responses to PcG in the three different strains of guinea pig assayed by PCA and ASA reactions are summarized in Table 1. In the group of Hartley guinea pigs that received PcG (10 mg/body $\times$ 10, sc), 160-fold, 40-fold and 5-fold PCA antibody titers were detected in 2, 2, and 2 out of 6 animals, respectively. Four out of the 6 animals showed severe positive ASA reaction (death by anaphylactic shock)

Table 1. Immune responses to benzylpenicillin potassium (PcG) in three guinea-pig strains detected by passive cutaneous anaphylaxis (PCA) and active systemic anaphylaxis (ASA) reactions.

Group	Strain	Eliciting antigen	Guinea pig No	PCA titer	Grade of ASA
Group 1 PcG 10 mg/body $\times$ 10, sc	Hartley	PcG-BSA	1	160	3 (D)
			2	160	3 (D)
			3	5	0
			4	40	3 (D)
			5	5	0
			6	40	3 (D)
Group 2 PcG 10 mg/body $\times$ 10, sc	Strain 2	PcG-BSA	7	40	3 (D)
			8	40	2
			9	40	2
			10	40	3 (D)
			11	40	2
			12	40	2
Group 3 PcG 10 mg/body $\times$ 10, sc	Strain 13	PcG-BSA	13	20	3 (D)
			14	20	3 (D)
			15	20	3 (D)
			16	<5	3 (D)

BSA: bovine serum albumin, D: death by anaphylactic shock.

scores of 3.

The PCA antibody titer for all animals in the group of Strain 2 guinea pigs that received PcG was 40. Two out of the 6 animals showed severe positive ASA reaction scores of 3. The PCA antibody titer for 3 of the animals in the group of Strain 13 guinea pigs that received PcG was 20, and that for the other animal in this group was less than 5. All animals of this group showed severe positive ASA reaction scores of 3.

Table 2 summarizes the immune responses to CET of the three different strains of guinea pigs obtained by PCA and ASA reactions. In the group of Hartley guinea pigs that received CET (20 mg/body $\times$ 12, sc), 160-fold, 40-fold and 10-fold PCA antibody titers were observed in 2, 3, and 1 out of 6 animals, respectively. Five out of the 6 animals showed strong positive ASA reaction scores of 3.

The PCA antibody titer for all animals in the group of Strain 2 guinea pigs that received CET was 20. No animals of this group showed

positive ASA reaction scores of 3. In the group of Strain 13 guinea pigs that received CET, 20-fold, 10-fold, 5-fold and less than 5-fold PCA antibody titers were observed in 1, 1, 1, and 2 out of 5 animals, respectively. Three out of the 5 animals showed severe positive ASA reaction scores of 3.

From the above data, the highest PCA reaction was observed in Hartley guinea pigs with the two antibiotics tested, and the lowest PCA reaction was seen in Strain 13 guinea pigs. The PCA reaction in Strain 2 guinea pigs was intermediate for both antibiotics.

Hartley and Strain 13 guinea pigs displayed high ASA reaction to the two antibiotics, while Strain 2 exhibited a relatively low ASA reaction. In general, the degree of the ASA reaction is associated with that of the PCA reaction in Hartley guinea pigs (Shinkai *et al.*, 1991; Verdier *et al.*, 1994; Nagami *et al.*, 1995). Although Strain 13 guinea pigs gave the lowest PCA antibody response to the two antibiotics, this strain displayed severe ASA reaction. Since

Table 2. Immune responses to cephalothin sodium (CET) in three guinea-pig strains detected by passive cutaneous anaphylaxis (PCA) and active systemic anaphylaxis (ASA) reactions.

Group	Strain	Eliciting antigen	Guinea pig No	PCA titer	Grade of ASA
Group 4 CET 20 mg/body $\times$ 12, sc	Hartley	CET-BSA	17	40	3 (D)
			18	160	3 (D)
			19	40	3 (D)
			20	160	3 (D)
			21	40	3 (D)
			22	10	2
Group 5 CET 20 mg/body $\times$ 12, sc	Strain 2	CET-BSA	23	20	2
			24	20	2
			25	20	2
			26	20	2
			27	20	2
			28	20	2
Group 6 CET 20 mg/body $\times$ 12, sc	Strain 13	CET-BSA	29	<5	3 (D)
			30	<5	2
			31	20	3 (D)
			32	10	3 (D)
			33	5	2

BSA: bovine serum albumin, D: death by anaphylactic shock.

the bronchia is the target tissue for the ASA reaction in guinea pigs, it may be speculated that the bronchia of this strain is more hypersensitive to anaphylactic reaction compared with the other two strains possessing different major histocompatibility complexes (Chiba and Sugimoto, 1983). As one fact supporting this view, Mikami *et al* (1990) have previously reported that inbred strains (Strain 2, Strain 13 and JY-1) have higher bronchial sensitivity to acetylcholine than the outbred strain (Hartley), and Strain 13 has the highest sensitivity among the four strains consequently. In this connection, other researchers have shown that strain differences in tracheal responsiveness are evaluated in two guinea-pig strains, and tracheal rings from the BFA strain develop a higher response to acetylcholine and barium chloride compared with the Dunkin-Hartley strain (Bidon *et al.*, 1995). Furthermore, Strain 13 guinea pigs are known as an immune disease model for allergic myositis (Matsubara and Takamori, 1987) and allergic encephalomyelitis (Karlik *et al.*, 1989; Gunn *et al.*, 1989). A different inbred strain, IMM/S 209, is also known as an asthma model with high sensitivity to respiratory anaphylaxis (Westphal-Petersen *et al.*, 1990; Winthereik *et al.*, 1992).

For both PCA and ASA reactions to the two antibiotics, individual variation was observed in Hartley guinea pigs; two animals (No.3 and No.5) showed low response to PcG compared with the other 4 animals. One animal (No. 22) also showed low response to CET compared with the other 5 animals. Such variation was found in outbred Hartley guinea pigs, but it was not found in inbred Strain 2 or Strain 13.

In our previous study, the differences in humoral and cellular immune responses to TNBS in three guinea-pig strains were investigated (Aida *et al.*, 1997), and the relative intensities of the humoral immune responses to TNBS and individual variation in Hartley guinea pigs were nearly in agreement with those obtained in the present study using antibiotics. In conclusion, it was clarified that the Hartley guinea pigs, which are the most common strain used in ordinary allergenicity tests for new drugs, was the highest responder to the two antibiotics in regard to PCA and ASA immunoreactions, although individual variation

was found in analytical data in this strain.

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