PENICILLIN G-INDUCED CUTANEOUS ANAPHYLAXIS IN THE GUINEA PIG

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Accepted August 16, 1989

Abstract.....Allergic cutaneous responses were induced by intradermal injection of penicillin G (PCG) and PCG-bovine serum albumin (BSA) conjugates onto the back of guinea pig actively immunized with PCG potassium (25 mg/animal) incorporated in Freund's complete adjuvant. The PCG-induced response was characterized macroscopically by erythema and edema with a maximum level at 24 hrs after elicitation and microscopically by the infiltration with basophils, macrophages and lymphocytes following with neutrophils. In addition, intensity of macrophage-infiltration shared a similar time course change with those of erythema and edema. These suggest that this response is associated to a delayed type hypersensitivity of Jones-Mote type. On the other hand, in the PCG-BSA-induced response the edema with erythema at the early phase was a noticeable observation and this response disappeared within 12 hrs, although the erythema continued by 24 hrs. Microscopically, the degranulation of mast cells and severe infiltration with neutrophils in the early phase and the infiltration of eosinophils in the late phase accompanying the infiltration with monocytes, basophils and lymphocytes were characteristic findings, which suggest that PCG-BSA response is a similar hypersensitivity to an atopic dermatitis. As mentioned above, we confirmed two types of allergic cutaneous responses in the guinea pig immunized with PCG.

Key words : Penicillin G, cutaneous anaphylaxis, guinea pig.

INTRODUCTION

Penicillin G (PCG) is a well known β -lactum antibiotics that induces severe allergies in human, but the antigenicity following immunization with PCG alone is scarcely reported in preclinical studies. In the first report, we preclinically studied the antigenicity of PCG and demonstrated that severe systemic anaphylaxis accompanying with production of humoral antibodies at high levels were induced in guinea pigs immunized with a high dose of PCG (Nagatahira et al., 1989). In this second paper, we have studied cutaneous anaphylaxis in the PCG-immunized guinea pigs.

MATERIALS AND METHODS

Animals : Hartley male guinea pigs were purchased from the JAPAN SLC Inc. (Shizuoka, Japan) and used in experiments after housing for at least 7 days in our laboratory (temperature 22 ± 2 °C, humidity $55 \pm 10\%$).

Immunization : Guinea pigs weighing 430–530g were immunized with PCG potassium (Meiji) 25 mg according to the methods of Nagatahira et al.(1989) and Magnusson and Kligman (1969). In the former method, 1 ml of PCG potassium incorporated in an Freund's complete adjuvant (FCA, Difco)-saline mixture (25 mg/ml) each on days 0, 7 and 21 was injected to a guinea pig : intramuscularly into both thighs (0.3 ml x 2) and intracutaneously into four locations (0.1 ml x 4) on the back. On the other hand, in the method of Magnusson and Kligman which is used for the maximization test, two intradermal injections (0.05 ml x 2) on the back of guinea pig were made with PCG potassium in saline (125 mg/ml), with PCG incorporated in an FCA-saline mixture (125 mg/ml) and with FCA alone. Six days later, the injected area was epidermally pretreated with 10% sodium dodecyl sulfate (Wako) in white vaseline 24 hrs before a 2 x 4 cm patch (Whatman No. 3MM filter paper) containing 0.2 ml of PCG in saline (125 mg/ml) was applied. The patch was covered by an overlapping tape for 48 hrs.

PCG-BSA conjugate : The PCG-bovine serum albumin (BSA) conjugate was prepared according to the method of Levine and Redmond (1968). PCG (3g) was mixed with BSA fraction V (Armour) (1g) and stirred in 100 ml of saline maintaining at pH 11.5 by addition of 1N NaOH (at room temperature). One hr later, the mixture was centrifuged at 10,000 r. p. m. for 30 min after adjusted at pH 4.0 with 1N HCl. The precipitates were dissolved in 150 ml saline, and then 1N NaOH was added to adjust at pH 9.0. The procedures as mentioned above were repeated three times to wash the precipitates. The final precipitates in saline were dialyzed against 1mM phosphate buffered solution (pH 7.2) for five days. This dialyzate was centrifuged at 10,000 r. p. m. for 30 min and the lyophilized supernatant was used as a PCG-BSA conjugate.

Cutaneous response : This response was elicited, two weeks after the last

PCG-induced cutaneous anaphylaxis

immunization, by intradermal injection (0.05ml) of saline containing 5% PCG or 5% PCG-BSA onto the shaved back. The intensity of the response was macroscopically scored at 1, 3, 6, 12, 24, 48 and 72 hrs after elicitation as follows :

Score 0 : no response

1 : slight erythema or edema

2 : scattered mild erythema or edema

3 : moderate erythema or edema

4 : severe erythema or edema

Microscopic examination : After macroscopic observation for cutaneous response, the animals were sacrificed by cutting abdominal aorta under ethyl ether anesthesia to flay their back skins. The sections from antigen-injected sites were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin or eosin-methylen blue for histopathologic examinations including cellular infiltrations or mast cell degranulation. The intensity of cellular infiltrations was scored as follows :

Score 0 : no response

1 : slight infiltration

2 : scattered mild infiltration

3 : moderate infiltration

4 : severe infiltration

RESULTS AND DISCUSSION

Allergic cutaneous responses were induced by intradermal injection of PCG or PCG-BSA onto the back of guinea pigs immunized three times with PCG potassium 25 mg incorporated in a FCA-saline mixture. In the early phase of the response elicited with the injection of PCG, no observation to be noted produced except for slight erythema and edema which might be due to nonspecific irritation of the injection. But from 6 hrs after elicitation, allergic erythema and edema appeared on PCG-injected sites and reached a maximum level at 24 hrs (Table 1). In addition, as shown in Fig. 1 this response was microscopically characterized by the infiltration with basophils and macrophages following with neutrophils and the score of infiltration with macrophages shared a similar time course change with the sumscore of erythema and edema. The infiltration with lymphocytes also was confirmed. These suggest that this PCG-induced cutaneous response might be the delayed type hypersensitivity. Although the delayed type hypersensitivity is known to include four types : tuberculin type, contact hypersensitivity type, Jones-Mote type (cutaneous basophil hypersensitivity : CBH type) and granulomatous type, the above response is postulated to be closely associated to Jones-Mote type (Mahapatro and Mahapatro, 1984) among these types because of the marked infiltration with basophils and having a maximum level 24 hrs after elicitation. This postulation complements with the report of Colvin et al. (1973). Namely, they have reported

Table 1. Macroscopic findings of PCG-induced cutaneous anaphylaxis in guinea pigs.

Finding	No. of animals	Score						
		1h	3h	6h	12h	24h	48h	72h
Flare	10	0.1 ±0.10	0.1 ±0.10	1.4 ±0.16	2.5 ±0.17	3.7 ±0.15	2.9 ±0.10	2.0 ±0.00
Wheal	10	0.1 ±0.00	0.0 ±0.00	0.8 ±0.29	1.4 ±0.34	3.2 ±0.42	2.2 ±0.20	1.3 ±0.24
Flare and Wheal	10	0.2 ±0.20	0.1 ±0.10	2.2 ±0.33	3.9 ±0.48	6.9 ±0.59	5.1 ±0.23	3.3 ±0.24

Each value indicates the mean $\pm$ S.E..

PCG-induced cutaneous anaphylaxis

Fig. 1. Microscopic findings in PCG-induced cutaneous anaphylaxis in guinea pigs.

that Jones-Mote type cutaneous response induced in guinea pigs immunized with human serum albumin was characterized by erythema from 6 hrs after elicitation and a maximum response with the infiltration with basophils and monocytes 24 hrs after. These were well consisted with the results demonstrated in this paper.

As mentioned previously, PCG is a medicament known as an allergen for human, and only a few report its antigenicity in preclinical models. One of them is a delayed-type hypersensitivity of PCG reported in maximization test of guinea pigs. So, we examined a time course change of PCG-induced cutaneous response in guinea pigs immunized according to the method of maximization test and the results were compared with those mentioned above. We confirmed that both cutaneous responses shared a similar time course change in sumscore of erythema and edema and microscopic observations (Table 2).

It is generally accepted that a low molecular compound could cause more easily and more potently allergic responses by conjugating with high molecular compounds such as proteins (Levine and Ovary, 1961 ; Torii and Kohoriuchi, 1961). Then, in this report, we also studied the cutaneous response induced by PCG-BSA of a protein conjugate antigen. The results showed that PCG-BSA-induced cutaneous response was much associated with a immediate type response and different from the typical delayed type response induced with PCG alone (Table 3). In the early phase, the edema with erythema was a noticeable observation, and this response decreased

Table 2. Macroscopic and microscopic findings of a modified maximization test.

Findings		Response score and histological score			
		1h	6h	24h	72h
Macroscopically ^{a)}	Flare	0.3 ±0.21	1.8 ±0.17	3.7 ±0.21	1.7 ±0.21
	Wheal	0.0 ±0.00	1.0 ±0.00	3.3 ±0.42	0.7 ±0.21
	Flare and Wheal	0.3 ±0.21	2.8 ±0.17	7.0 ±0.63	2.3 ±0.42
Microscopically ^{b)} (Cellular infiltration)	Basophil	0	0	3	4
	Neutrophil	1	2	3	1
	Macrophage	1	2	3	3
	Lymphocyte	0	0	1	2
	Eosinophil	0	0	1	2

Each value indicates the mean ± S.E. of 6 animals^{a)} or the mean of two representative animals^{b)}.

Table 3. Macroscopic findings of PCG-BSA-induced cutaneous anaphylaxis in guinea pigs.

Finding	No. of animals	Score						
		1h	3h	6h	12h	24h	48h	72h
Flare	3	2.7 ±0.33	3.0 ±0.00	2.3 ±0.33	2.7 ±0.88	3.0 ±0.58	1.3 ±0.33	1.0 ±0.11
Wheal	3	3.0 ±0.00	2.0 ±0.58	1.0 ±1.00	0.0 ±0.00	0.3 ±0.33	0.0 ±0.00	0.0 ±0.00
Flare and Wheal	3	5.7 ±0.33	5.0 ±0.58	3.3 ±0.88	2.7 ±0.88	3.3 ±0.88	1.3 ±0.33	1.0 ±0.00

Each value indicates the mean ± S.E..

gradually with time and disappeared within 12 hrs, although the erythema continued by 24 hrs. Fig. 2 indicates the microscopic findings. The degranulation of mast cells (Photo. 1) (Parish, 1972) and severe infiltration with neutrophils in the early phase and the infiltration of eosinophils in the late phase were characteristic findings, though the infiltration with monocytes and basophils also were observed. Especially, the degranulation of mast cells suggests that the PCG-BSA-induced

PCG-induced cutaneous anaphylaxis

Fig. 2. Microscopic findings in PCG-BSA-induced cutaneous anaphylaxis in guinea pigs.

Photo. 1. Photographic profile of cutaneous mast cell (x1050). (A) : PCG-BSA-injected site, (B) : uninjected site.

cutaneous response is in great relation to type I allergy caused by homocytotropic antibody. The previous report (Nagatahira et al., 1989) which revealed that homocytotropic antibody was detected at high levels in sera of guinea pigs immunized with PCG according to the method of this paper supports this postulation. In addition, these characteristics seems to be similar to those of atopic dermatitis of a type I allergy. Thus, in cutaneous responses of atopic patients, erythema and edema appear following intradermal injection of corresponding antigens and disappear within 24 hrs after duration of 6–12 hrs. While the microscopic picture shows the degranulation of mast cells and the infiltration of monocytes, neutrophils and eosinophils (Dolovich et al., 1973; Lemanske and Kaliner, 1981; Solley et al., 1976).

As mentioned above, we confirmed allergic cutaneous responses in guinea pigs immunized with PCG and demonstrated that the cutaneous response of a Jones-Mote type or an atopic dermatitis type was induced by intradermal injection of PCG or PCG-BSA, respectively. For the future, the pathogenic mechanism and the efficacy of drugs for these allergic cutaneous responses will be studied to apply as a preclinical approach for the antigenicity of β -lactum antibiotics like PCG.

ACKNOWLEDGEMENT

We are grateful to Ms. Y. Nakajima for preparing the manuscript.

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