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PRODUCTION OF RAT HOMOCYTOTROPIC ANTIBODIES USING LOW DOSE, LONG TERM ORAL EXPOSURE TO PENICILLIN G

By

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It is known that penicillin residues are found in milk and meat products as well as several other sources (1, 2, 8). It is possible that exposure of humans to these sources may produce IgE-type antibodies, as individuals could be exposed to low doses of penicillin residues over long periods of time. It was shown by Levine et al. (3) that in mice, injections of small amounts of penicillin conjugates over long periods is efficacious for producing homocytotropic antibodies in these animals.

The present study was undertaken to determine whether low dose, long term oral exposure to penicillin G (Pen-G) in rats can induce IgE and IgGa antibody production. Indeed, the addition of 0.1 to 1 U/ml of Pen-G to the drinking water of rats over several months caused the production of both IgE and IgGa antibodies in these animals, suggesting that the oral route may be effective in sensitizations in man.

MATERIALS AND METHODS

Human Sera

Sera from 15 individuals with reported systemic reactions to Pen-G as well as eight non-allergic volunteers were used. The sera were stored at -70°C for up to 2 months.

Pen-G (Sigma Chem. Co., St. Louis, Mo.) was used in the rat mast cell test (RMCT) (5) at concentrations ranging from 0.1 to 10,000 U/ml as described

below. The concentration of Pen-G employed in the oral exposure of rats is indicated in the following:

Oral Exposure of Rats to Pen-G

Two groups of six rats were exposed to Pen-G in their drinking water at concentrations of 0.1 and 1 U/ml for 1 month, each rat being exposed to between 60 and 600 U Pen-G/month. Two other sets of rats including the appropriate control group were treated in a similar manner for 2 and 3 months, respectively. All rats were bled 2 weeks after oral exposure to Pen-G had been stopped. Six other rats were orally treated with 1 U/ml of Pen-G for 1 month, injected subcutaneously with 50,000 U Pen-G 2 weeks later, and bled after an additional 2 week-interval. A control group of six rats was treated in a similar manner except for the omission of Pen-G from their drinking water.

Rat Mast Cell Technique (RMCT)

A. Detection of IgE antibodies in the sera of penicillin-allergic individuals using the RMCT: The RMCT for the detection of human IgE-type antibodies was performed as previously described (5). Briefly, 0.05 ml aliquots of allergic serum, antigen, normal rat serum and mast cell suspension were incubated at 37° C for 3 min. After the incubation period, a 2 per cent glutaraldehyde solution in 0.2 M sodium phosphate buffer, pH 7.3, was added to the cell mixture. After 60 min at 4° C, the cell mixture was spun in a PR2 International centrifuge at 300 g for 5 min at 4° C. A 0.05 ml aliquot of the resultant pellet was placed on a coverslip and smeared with a second coverslip. After the smear had been air-dried, the coverslip was dyed with Wright dye, washed and mounted on a microscope slide as described previously (5). The mast cell changes were observed under the microscope and the results expressed as the percentage of morphologically altered cells compared to total cell population.

B. Detection of rat IgGa and IgE antibodies in the sera of rats treated with penicillin-G: Two types of homocytotropic antibodies have been recognized in the rat. One type (IgE) is characterized by its firm binding to target cells. It requires between 1 to 2 h of *in vitro* incubation to sensitize normal rat mast cells and is not removed by washing (IgE and IgGa antibodies mediate release of histamine from rat peritoneal cells). The second homocytotropic antibody (IgGa) is characterized by its weak binding to target cells and is assessed by incubating normal rat mast cells with the appropriate serum and antigen for a short incubation time. To determine IgE antibodies, suspension of normal rat mast cells (0.05 ml) were incubated with the test serum (0.05 ml) for 1 h at 37° C. Then the cells were washed with medium 199 and subsequently resuspended in medium 199 and challenged with Pen-G for 10 min. To determine IgGa antibodies, an aliquot of 0.05 ml of rat mast cell suspension was incubated with an equal amount of rat serum and Pen-G at 37° C for 5 min. Morphological changes in the rat mast cells were evaluated as indicated in (A).

C. Determination of the sensitization of rat mast cells from orally treated animals: Mast cell suspension from orally treated animals was obtained from the peritoneal cavity of the rat, washed with medium 199 and challenged with Pen-G at concentrations indicated in the results section. The presence of cell bound IgE antibodies was indicated by morphological changes in the mast cells on challenge with Pen-G.

RESULTS

Table 1 summarizes the rat mast cell responses of sera from 15 patients having had immediate-type reactions to penicillin anytime from 2 weeks to 22 years previously. These responses indicate the presence of IgE antibodies in the sera. It is interesting to note that five patients (# 11 to 15) had had reactions to penicillin 15 to 22 years previously without knowingly being re-exposed to this drug, and their sera gave positive responses in the RMCT. To explore the possibility that a non-

TABLE 1
RMCT Responses of Sera from Penicillin Allergic Individuals.

Patient	Type of reaction*	Time from allergic reaction to obtention of serum samples	Conc. of Pen-G** giving optimal responses U/ml
C.W.	U, An	2 weeks	50
T.H.	As, U	2 weeks	50
C.M.	U	3 months	500
B.W.	U	6 months	1000
L.T.	E, An	1 year	500
R.R.	U, An	2 years	500
R.J.	U, An	2 years	1000
B.B.	As, U	2 years	1000
E.A.	U	4 years	4000
D.F.	U	5 years	2000
A.D.	U, An	15 years	5000
E.B.	U, An	15 years	2000
R.F.	U, An	22 years	2000
F.Q.	As, U, An	19 years	2000
E.R.	U	20 years	1000

* U = urticaria, An = angiodema, As = asthma, E = eczema.

** The eight non-allergic controls did not give responses in the RMCT.

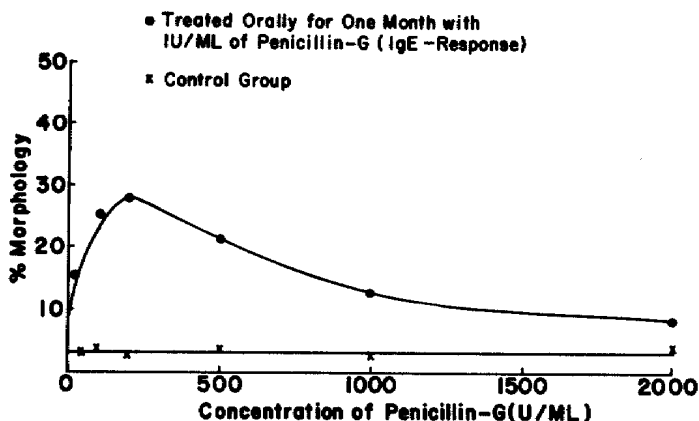


Fig. 1.

Detection of IgE antibodies present in a rat orally exposed to penicillin G.

therapeutic exposure to penicillin may have induced continued sensitization in these patients, an animal model system was developed to explore the possibility that low dose, long term oral exposure to penicillin G is capable of producing antibodies in these animals. For this purpose, rats were given penicillin G in their drinking water at very low levels (0.1–1 U/ml) and these animals were examined for the presence of IgE and IgGa antibodies after various periods of exposure to the drug. Figs. 1 and 2 show a typical dose response curve of serum from a rat exposed to Pen-G in the manner described above for 1 month, and the sera tested for IgE (Fig. 1) and IgGa (Fig. 2) antibodies, respectively, by the RMCT. Significant responses were obtained at concentrations of Pen-G ranging from 5–500 U/ml for both types of antibodies, whereas no response was obtained for the control group. Table 2 summarizes the detection of IgE and IgGa antibodies of rats orally treated with Pen-G for 1, 2 and 3 months, respectively. As early as after 1 month of exposure, IgE antibodies were detected in the sera of the treated animals, and in a separate group of rats, IgE was found in the sera after 3 months of

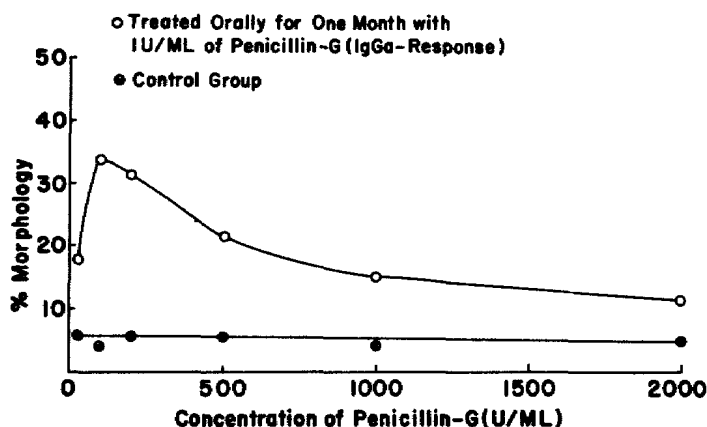


Fig. 2.

Detection of IgGa antibodies present in a rat orally exposed to penicillin G.

TABLE 2

Detection of IgE and IgGa Antibodies in Rats Orally Treated with Penicillin-G Using the RMCT.*

	No. of rats in group	No. of rats actively sens.	No. of rats with	
			IgE	IgGa antibodies
Exposed group				
1 month	5	3	4	5
2 months	6	6	6	6
3 months	6	ND	6	6
Non-exposed group				
1 month	6	0	0	
2 months	6	0	0	
3 months	6	ND	0	

* Rats were treated with between 0.1 to 1 U/ml of Pen-G in their drinking water.

continuous exposure. In addition, mast cells removed from the peritoneal cavity of the treated rats, were found to be actively sensitized to Pen-G as morphological changes in rat mast cells occurred in the presence of Pen-G, whereas cells from the un-

treated group did not respond to Pen-G. Similar results were obtained when sera of treated rats were tested for the presence of IgGa antibody.

Having established sensitization in the rat by oral exposure, it was desirable to determine the effect of previous oral exposure on the efficacy of inducing antibody production in animals by a single intramuscular injection of Pen-G. Thus, six rats were orally treated with 1 U/ml Pen-G for 1 month followed by an intramuscular injection of 50,000 U Pen-G. Five of these rats produced IgE and IgGa antibodies in which responses were obtained in the RMCT at 5 U/ml or less of Pen-G. In the control group that had been injected only with Pen-G without previous oral exposure (five rats), three sera responded at higher levels of Pen-G (> 5 U/ml) indicating that lower levels of IgE and IgGa antibodies were produced. These results indicated that low dose oral exposure of Pen-G not only produces IgE and IgGa antibodies in rats but that such treatment causes significant increase in the levels of those antibodies in animals given a single injection of Pen-G.

DISCUSSION

Despite intensive research during the last 10 years on the mechanism of penicillin allergy and the possible ways to prevent it, penicillin allergy remains an important problem in clinical practice, and penicillin is without doubt the antibiotic most frequently causing hypersensitivities. According to Barrett-Conner (1), 5 per cent of the total population of the USA is sensitive to penicillin. In an independent review of this problem an incidence range of 0.1–10 per cent is reported (2, 6, 8). The high rate of sensitization to penicillin may be due not only to the frequency of therapeutic use, but also to environmental exposure (vaccine, milk, milk products, meats, meat products, tooth paste, cosmetics) (2). In addition, penicillin patients are known to maintain their sensitivity for years after their therapeutic exposure. In 1969, in a survey performed by the Food and Drug Directorate, in Ottawa, Can-

ada, it was found that roughly 2 per cent of milk samples tested microbiologically contained Pen-G prior to pasteurization. In the present study, it was shown that long term oral exposure is effective in causing sensitization in rats as evidenced by the production of IgE and IgGa antibodies. Recently, these results were confirmed in a separate study (4).

The results of the present study, using the animal model system, suggest that a significant percentage of the population may become sub-clinically sensitized to penicillin by non-therapeutic exposure and/or therapeutic exposure (oral or injection). In this connection, it was found that rats maintained on low dose, long term oral exposure showed a higher degree of sensitization to an injection (50,000 U) of Pen-G compared to a control group of rats injected with the same dose of Pen-G.

There are reports of individuals who on the first therapeutic exposure to the drug showed symptoms of sensitization. In fact, a recent report by Spark (7) showed that a fatal anaphylactic reaction due to oral penicillin occurred at a first therapeutic exposure. It would appear that the presence of Pen-G as a drug residue in foods, primarily milk, could be an important factor in sensitizing the population to the drug and suggests that more extensive screening of foods for the presence of penicillin residue should be carried out.

To date, the microbiological technique, because of its sensitivity, is the principal method used for the detection of penicillin in milk. However, there is little evidence that the concentrations or the forms of penicillin detected represent allergenically active forms of penicillin. More work should be carried out to detect the allergenic properties of penicillin residues that exist in various foods, cosmetics and vaccines. Moreover, methods for the detection of IgE antibodies such as the RMCT and radioallergosorbent test (RAST) should be used to detect the penicillin residues.

SUMMARY

Sera from 15 patients with immediate hypersensitivity reactions to penicillin G gave positive responses in the rat mast cell test (RMCT) indicating the presence of IgE-type antibodies in the sera. Five sera were from patients who had had reactions to penicillin 15 to 22 years previously without known re-exposure to this antigen. To explore the possibility that non-therapeutic exposure to penicillin may have produced continued sensitization in these patients, an animal model system was developed to explore the efficacy of low dose, long term oral exposure to penicillin G in rats for producing homocytotropic antibodies in these animals. It was found that when rats were given penicillin G in their drinking water at concentrations of 0.1 to 1 U/ml over a period of 1 to 3 months they produced serum IgE and IgGa antibodies. In addition, IgE antibodies were actively bound to the peritoneal mast cells of these animals. The presence of circulating or cell bound antibodies was detected using the rat mast cell test. It was also shown that rats given penicillin G orally for 1 month were more prone to antibody production after a single intramuscular injection of penicillin G compared to a control group receiving only the intramuscular injection of this antigen. The results of this study are discussed in terms of possible non-therapeutic sensitization towards penicillin G in the human population.

REFERENCES

1. Barrett-Conner, E. (1970): Untoward reactions to antibacterial drugs. *Mod. Med.* 38, 101-105.
2. Girard, J. P. (1971): Allergic reactions to antibiotics. *Helv. med. Acta* 36, 3-17.
3. Levine, B. B. (1971): Atopy and mouse models. *Int. Arch. Allergy* 41, 88-92.
4. Liakopoulou, A. & Perelmutter, L. Unpublished observations.
5. Perelmutter, L. & Millard, E. (1971): A modified rat mast cell technique for the detection of human IgE-type reagins. *Int. Arch. Allergy* 40, 675-681.
6. Smith, T. J. (1964): *Diseases of medical progress*. p. 360. C. C Thomas, Springfield, Ill.

7. Spark, R. P. (1971): Fatal anaphylaxis due to oral penicillin. Amer. J. clin. Path. 56, 407-410.
8. Steward, T. G. (1968): Allergenic factors in penicillins and cephalosporins. Amer. Heart J. 75, 429-435.

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