Cancer Chemother Pharmacol (1989) 23: 333–336

Cancer Chemotherapy and Pharmacology

Short communication

Occurrence of an anti-peplomycin IgE antibody cross-reacting with bleomycin in a patient with cervical uterine cancer

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Summary. An allergic reaction to peplomycin was observed in a patient with cervical uterine cancer who had previously been treated with peplomycin. A positive Prausnitz-Küstner test and its elimination after heat treatment of the serum showed the production of anti-peplomycin IgE antibody. Peplomycin was coupled to a paper disc and a sensitive radioallergosorbent test for peplomycin was developed to quantitate the antibody. Patient serum IgE and IgG were purified by DE52 column chromatography; the IgE fraction contained binding activity to peplomycin. A competition test revealed that the antibody bound to both peplomycin and bleomycin. DNA, RNA, and mononucleotides had no effect on antibody binding, but the antibody inhibited peplomycin's activity.

Introduction

Bleomycin is an antibiotic polypeptide isolated from *Streptomyces verticillus* [10] that is effective in the treatment of squamous cell carcinomas, lymphomas, and testicular carcinomas [2]; its major side effect is pulmonary toxicity [2]. Peplomycin is a new derivative of bleomycin with low pulmonary toxicity and effective antitumor potency [7, 8]. To our knowledge, anti-peplomycin or -bleomycin antibody production in cancer patients as a side effect has not previously been reported. The present report describes the first case of allergic reaction to peplomycin in a patient with uterine cancer. The anti-peplomycin antibody found in patient serum was purified and characterized using radioallergosorbent tests.

Materials and methods

Patient. A 74-year-old woman with third-stage cervical uterine cancer was treated in Hamamatsu Medical Center from December 1982 until May 1983. The patient had received cobalt-60 radiotherapy (4,900 rad) and chemotherapy with both bleomycin (100 mg) and peplomycin (50 mg). Bleomycin was given as a suppository, and 10 mg peplomycin was injected weekly in the parametrium and cervix uteri. The patient denied a history of asthma and was not known to be allergic to any other drugs. She was admitted in November 1983 and treated for a relapse of uterine cancer until May 1984, receiving further cobalt-60 radiotherapy (4,020 rad) and chemotherapy with peplomycin (50 mg) locally injected in the parametrium. Cancer cells disappeared after these therapies. In January 1985, the patient was again admitted due to a relapse. Infiltration to the urinary bladder was not observed; chest X-ray revealed no metastatic tumor or pulmonary toxicity. On May 17, 1985, peplomycin chemotherapy was begun. As local injection was started, the patient had a sudden onset of nausea, dyspnea, and hypotension and became unconcious; the allergic reaction resolved within 15 min. A prick test of 0.4 μg against peplomycin was positive. The patient died of uremia in June 1986, 1 year after the allergic reaction.

Materials. Enzyme immunoassay kits for the determination of human IgE and antigen-specific IgG antibody were obtained from Boehringer (Mannheim) and Shionogi (Tokyo). ^{125}I-anti-(human IgE) antibody was obtained from Daiichi Radioisotope (Tokyo), and DE52, from Whatman (Maidstone). Bleomycin and peplomycin were received from Nihonkayaku (Tokyo). DNA was extracted from a cell line (Hos) derived from human osteosarcoma; labelling of the human DNA was carried out by a nick translation using [α-^{32}P]-CTP [6]. Control serum was obtained from a woman with no past history of allergy and a negative prick test against peplomycin.

Prausnitz-Küstner test. Patient serum (0.1 ml), control serum (0.1 ml), and physiological saline (0.1 ml) were injected i.d. over the antebrachium of a normal woman. The sensitized site was challenged 24 h later by the injection of 0.1 ml peplomycin (0.4 μg). Sera heated at 56° C for 30 min to destroy the receptor-binding site of IgE were also examined [3].

Ouchterlony immunodiffusion test. Double immunodiffusion was carried out on 2% agarose gel for 24 h at room temperature. The center well contained the patient serum (10 μl); the outer wells contained the peplomycin (10 μl) at 2, 1, 0.5, 0.1, and 0.05 mg/ml, respectively.

Radioallergosorbent test for peplomycin. Coupling of peplomycin to a CNB-activated paper disc was developed by a modification of the method of Wide et al. [11]. The peplomycin-paper disc was reacted with the sample (50–100 μl) in a tube at room temperature for 12 h , and

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the excess antibody was washed away. A total of 50 μl ^{125}I-anti-(human IgE) antibody (about 30,000 cpm) was added to the peplomycin-paper disc in a tube. The mixture was further incubated at room temperature for 12 h, and the radioactivity bound to the paper disc was measured in a gamma counter.

Degradation of DNA by peplomycin. The assay for the degradation of DNA by bleomycin, described previously [5], was used for peplomycin. The reaction mixture (50 μl) contained 10 μ*M* ^{32}P-Hos-DNA (12,000 cpm), 50 μ*M* sodium phosphate buffer (pH 7.0), 0.1 m*M* $FeSO_4$–$(NH_4)_2SO_4$, and peplomycin (0.4–12 μ*M*) in the presence or absence of anti-peplomycin IgE antibody.

Results

Prausnitz-Küstner reaction

Following peplomycin challenge, the recipient revealed an erythematous dermal reaction measuring 35 mm. The control serum and physiological saline were negative. Heat treatment of the patient serum resulted in the elimination of the Prausnitz-Küstner reaction. These results indicated that the allergic reaction against peplomycin was due to IgE. The IgE level of the patient serum was elevated to 296 IU/ml at allergic reaction. IgE levels at 6 and 12 months after the allergic reaction were 197 and 61 IU/ml, respectively.

Anti-peplomycin IgE antibody

The patient serum showed high activity for binding ^{125}I-anti-(human IgE) antibody to the peplomycin-paper disc; the dose-response curve of the binding activity is shown in Fig. 1. The control serum with the negative prick test showed no binding activity. However, the patient serum did not form a precipitin line in an Ouchterlony immunodiffusion test, and the passive hemagglutination test after the conjugation of sheep red blood cells and peplomycin was also negative.

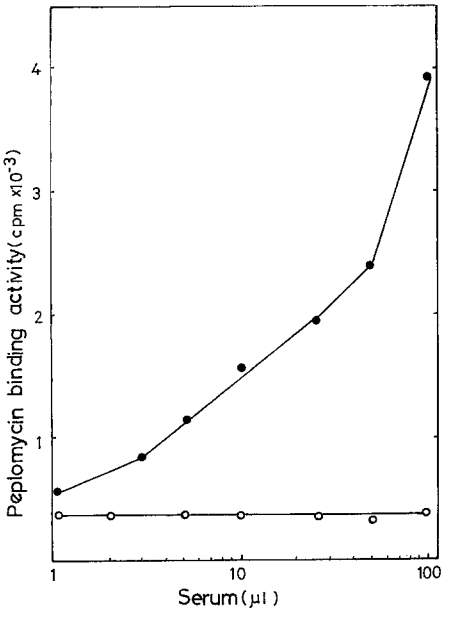

Fig. 1. Peplomycin-binding activity of patient serum. Patient serum, (●); control serum, (○)

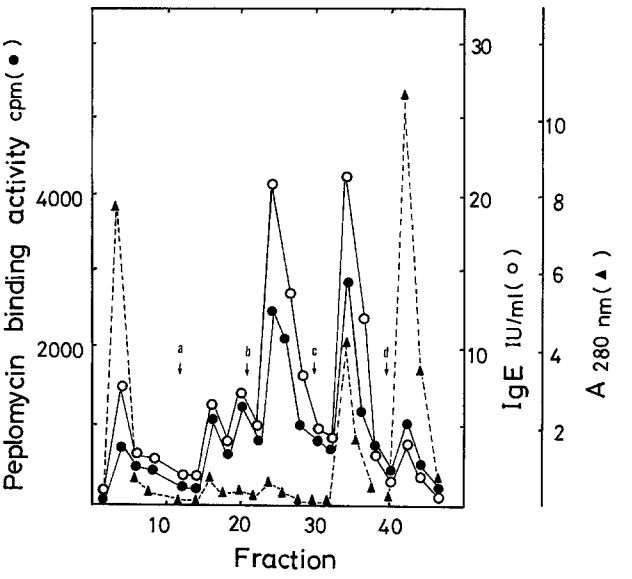

Fig. 2. DE52 column chromatography of IgE. The dialyzed sample (7 ml) of patient serum after ammonium sulfate fractionation was applied to a DE52 column (1 × 12 cm) equilibrated with 40 m*M* TRIS-HCl (pH 8.0). Stepwise elution was carried out as indicated by *arrows*: *a*, 70 m*M*; *b*, 100 m*M*; c, 200 m*M*; and *d*, 400 m*M* TRIS-HCl (pH 8.0); 3-ml fractions were collected. Peplomycin-binding activity; (●); IgE, (○); and A_{280}, (▲)

Purification of the anti-peplomycin IgE antibody

Patient serum (6 ml) was fractionated by precipitation with 60% saturated ammonium sulfate; after centrifugation, the precipitate was dissolved in 5 ml 40 m*M* TRIS-HCl buffer (pH 8.0). The protein solution was dialyzed against the same buffer and then subjected to DE52 column chromatography; the elution profile is shown in Fig. 2. IgE was recovered in two peaks, with an overall recovery from the original serum of 40%. Peaks 1 and 2 were each eluted in

Table 1. Specificity of anti-peplomycin IgE antibody

Inhibitors (20 μ*M*)	Binding (%)
Control	100
Bovine serum albumin (1 mg/ml)	95
Peplomycin lot U0104BS[a]	10
Peplomycin lot 01020S19[a]	14
Bleomycin	42
Phenylalanine	88
AMP	82
GMP	93
CMP	101
TMP	101
UMP	98
DNA (10 μg/ml)	90
RNA (10 μg/ml)	88
Poly (A) (10 μg/ml)	107

[a] Lot U0104BS is purified peplomycin. Lot 01020S19, containing a stabilizer, phenylalanine, was used clinically in the patient as well as for radioallergosorbent tests

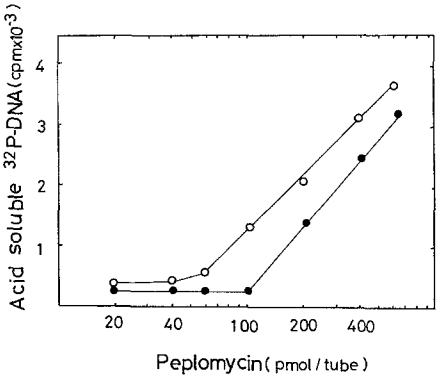

Fig. 3. DNA degradation by peplomycin in the presence of anti-peplomycin IgE antibody. The anti-peplomycin IgE antibody, fraction 25 (●, 0.4 IU), and fraction 32 (○, 0.05 IU) of DE52 column chromatography were used. Degradation of DNA was determined as acid-soluble radioactivity from [^{32}P]-Hos-DNA. DNA degradation in the presence of 0.05 IU IgE was almost the same as that in its absence.

100 m*M* and 200 m*M* TRIS-HCl buffer (pH 8.0). IgG was eluted as a single peak in 40 m*M* buffer (data not shown). The elution profile of the peplomycin-binding activity corresponded to the two peaks of IgE; the IgG fraction did not show the binding activity of enzyme-linked anti-(human IgG) antibody to the peplomycin-paper disc. These data indicate that the antibody to peplomycin in patient serum is IgE antibody. The IgE of peak 1 was purified 13-fold from the serum fraction, and the specific activity was 26 IU/mg protein; peak 1 was used for the following experiments.

Specificity of the anti-peplomycin IgE antibody

To determine the specificity of the anti-peplomycin IgE antibody, bleomycin and peplomycin were examined as to their ability to compete with peplomycin in the radioallergosorbent test. As shown in Table 1, both purified and clinically used peplomycin at 20 μ*M* inhibited the binding activity of the antibody to 10% of the control values. Bleomycin, which is structurally different from peplomycin at the terminal amine moiety, was also a good competitor and inhibited the binding to 42%. These data suggest that antigenic determinants of peplomycin reside in the common bleomycinic acid moiety of peplomycin and bleomycin. As bleomycin is a DNA-binding protein, the effect of nucleotides on the interaction between peplomycin and anti-peplomycin antibody was also examined: DNA, RNA, and poly (A) at a concentration of 10 μg/ml as well as various mononucleotides had no effect.

Inhibition of peplomycin activity by the anti-peplomycin antibody

To evaluate the therapeutic effect of the anti-peplomycin antibody on chemotherapy, DNA degradation by peplomycin was measured in the presence of different concentrations of IgE. The dose-response curve of peplomycin activity is shown in Fig. 3. DNA degradation in the presence of 0.05 IU IgE was linear, with the concentration of peplomycin, from 50 to 500 pmol/tube. The degradation of DNA by 100 pmol peplomycin was 1432 cpm (43 pmol/30 min, about 9% of the total DNA in the reaction mixture. However, in the presence of 0.4 IU IgE, DNA degradation by 100 pmol peplomycin was completely inhibited. This peplomycin activity was observed even in the presence of 0.4 IU IgE by increasing the peplomycin concentration. The control IgE (0.4 IU) had no effect on peplomycin activity. This data shows that 1 IU of the patient IgE inactivates 250 pmol peplomycin activity.

Discussion

From 1980 to 1985 there were 115 cases of cervical uterine cancer (squamous cell carcinoma) at the Hamamatsu Medical Center. Of these 43 patients were inoperable and were given both radiation and chemotherapy with either bleomycin or peplomycin. These drugs were given as a suppository via the vaginal cavity or by direct local, i.v., or i.a. injection. The total dose of bleomycin and peplomycin in these cases ranged from 50 to 300 mg, including the suppositories. The primary side effect was transient fever. Severe pulmonary fibrosis was not observed at these doses, and an allergic reaction was observed only in one case.

There have been only two reports on the experimental production of anti-peplomycin antibody; in these reports, peplomycin was conjugated to either mercaptosuccinylated bovine serum alvumin (BSA) or BSA [1, 4]. In the present case, it is possible that peplomycin was also conjugated in vivo with some endogenous protein during the second chemotherapy. In previous studies, the anti-peplomycin antibodies produced in animals were monospecific to peplomycin and did not cross-react with bleomycin, and their immunoglobulin class was not examined [1, 4]. However, in the present study the patient anti-peplomycin antibody was IgE-specific and revealed cross-reaction with peplomycin and bleomycin, as shown in Table 1. It has long been known that bleomycin possesses a DNA-binding site and that pyrimidines are preferentially released from DNA after the bleomycin treatment [9]. However, nucleotides did not interfere with the binding between peplomycin and the antibody, suggesting that the antigenic determinant and DNA-binding sites of peplomycin are different from each other. On the other hand, DNA degradation was suppressed by the anti-peplomycin antibody. IgG or IgE antibody production against these peptide drugs might be a cause of drug resistance in cancer patients.

Particular attention has been paid to pulmonary toxicity as a side effect of bleomycin and peplomycin. Blum et al. [2] have reported four cases of acute, fulminant side effects and five cases of milder, acute side effects due to bleomycin in 808 cancer patients. The etiology of this acute reaction has not yet been elucidated; some of these cases might have been due to allergic reaction by the IgE antibody, as described in this report.

Acknowledgements. We would like to thank Dr. A. Usami for technical assistance with peplomycin coupling and Prof. S. Ichiyama, Department of Biochemistry, Hamamatsu University, for his helpful comments and criticism.

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Received June 30, 1988/Accepted October 13, 1988