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Fresh *vs* aged benzylpenicillin on non-IgE responses in mice

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KEY WORDS penicillin G; ampicillin; piperacillin; IgG; IgM; IgA; enzyme-linked immunosorbent assay; haptens; cross reactions; inbred BALB c mice

AIM: To study whether or not the freshly prepared benzylpenicillin could induce different non-IgE antibody response from aged benzylpenicillin. **METHODS**: Antibody response was determined by enzyme-linked immunosorbent assay (ELISA). Antigen molecules recognized by antibodies and antigenic cross reactions were tested by hapten inhibition assay. **RESULTS**: Isotypes of specific non-IgE antibodies induced by freshly prepared benzylpenicillin were mainly IgM, and then IgG and IgA. Some parts of specific antibodies recognized benzylpenicillin molecule and major parts combined with degraded or transforming products. Isotypes of antibodies responsible for cross reaction were mainly IgG between benzylpenicillin and ampicillin and IgM between benzylpenicillin and piperacillin. **CONCLUSION**: Freshly prepared and aged benzylpenicillin induced different non-IgE antibody response.

Although < 10 % of all adverse drug reactions are immunologically mediated, they often cause the severest clinical symptoms, with 1 in 10 000 resulting in death[1-3]. Penicillins are the most frequently cited cause of antibiotic-induced allergic reactions[4,5]. However, our knowledge of the role of non-IgE antibodies such as IgG, IgM, and IgA against penicillins in adverse drug reactions remains limited. Serum non-IgE antibodies from mice recognized degraded products, polymer or transforming products, but not benzylpenicillin molecule[6], while at least some of sera from children combined the benzylpenicillin molecule itself[7]. We therefore have hypothesized that the difference of antibody response in human and in mice might be due to the fact that children were injected with freshly prepared benzylpenicillin while mice were immunized with aged benzylpenicillin. This study was to investigate whether or not the freshly prepared benzylpenicillin could induce different non-IgE antibody response from aged benzylpenicillin.

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MATERIALS AND METHODS

Reagents Horseradish peroxidase-labelled sheep anti-mouse IgG, IgM, and IgA were obtained from Gibco BRL (USA); bovine serum albumin (BSA) from Sigma Chemical Co (USA); protein 15 kDa of *Campylobacter jejuni* from Shanghai Institute of Immunology, 96-well polystyrene microtitre plates from Coster (USA). Benzylpenicillin, ampicillin, and piperacillin were obtained from Shanghai No 3 Pharmaceutical Factory.

Immunization BALB c mice (♀, aged 8 wk, weighing 22.5 ± s 2.3 g) were purchased from the Department of Experimental Animals, Shanghai Medical University. They were divided into 2 groups, 10 in each. Benzylpenicillin was dissolved in physiologic saline and protein 15 kDa of *C jejuni* was added into solution to 5 $mg \cdot L^{-1}$. The mice in experimental group were immunized with ip benzylpenicillin 5400 μg (0.2 mL/mouse) daily from d 1 to d 6, while the mice in control group were injected ip protein 15 kDa of *C jejuni* 5 $mg \cdot L^{-1}$ in saline. Serum samples were collected on d 10 and stored at −20 ℃.

ELISA The assay was performed[7]. Briefly, microtitre plates were coated with benzylpenicillin-BSA conjugates. After washing, diluted sera (1:30) were added to wells in triplicate and then incubated. The plates were washed, horseradish peroxidase-labelled sheep anti-mouse IgG, IgM, or IgA were added to wells and incubated. After washing, the substrate solution was added to wells and incubated. The absorbance (A) was read at 492 nm by EIA Reader (Bio-Rad, USA). A serum sample was considered to be positive when the A value of the serum tested was $> \bar{x} \pm 3s$ of the negative control sera. The antibody level was expressed as enzyme index.

Hapten inhibition assay The assay was performed[7]. Briefly, 50 μg of an antibody positive serum (1 : 15) was preincubated with 50 μL of freshly prepared benzylpenicillin,

ampicillin, piperacillin solutions, or aged corresponding solutions which had been stored at 37 ℃ for 24 h. ELISA was then performed as above.

RESULTS

Antibody responses induced by fresh benzylpenicillin ELISA showed that 50 %, 30 %, 20 % of experimental mice were positive for IgM, IgG, and IgA, respectively. The enzyme index of antibodies in serum positive mice or even in immunized mice were higher than control mice (Tab 1).

Comparison of inhibitory effect between fresh and aged benzylpenicillin The inhibitory % of aged benzylpenicillin on IgM, IgG, and IgA were higher than those of freshly prepared benzylpenicillin at 10 – 1000 μg. The order of inhibition appeared to be aged benzylpenicillin on IgG > IgM > IgA > fresh benzylpenicillin on IgG > IgM > IgA (Fig 1).

Tab 1. Specific non-IgE antibody responses in BALB c mice on d 10 induced by freshly prepared benzylpenicillin. $n = 20$, $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Antibodies	Enzyme index of antibodies		
	Control	Immunized	Positive
IgM	70 ± 11	93 ± 23[c]	113 ± 8[c]
IgG	59 ± 14	90 ± 26[c]	128 ± 9[c]
IgA	55 ± 15	64 ± 24	104 ± 10[c]

Cross reactions between benzylpenicillin, ampicillin, and piperacillin The inhibitory % of freshly prepared ampicillin solution on IgM, IgG, and IgA were higher than those of aged solution at 10 – 1000 μg. The order of inhibition appeared to be fresh ampicillin on IgG > aged ampicillin on IgG > fresh ampicillin on IgM > fresh ampicillin on IgA > aged ampicilin on IgM > aged ampicillin on IgA (Fig 1).

Contrary to ampicillin, the inhibitory % of freshly prepared piperacillin on IgM, IgG, and IgA were lower than those of aged piperacillin at 10 – 1000 μg. The order of inhibition appeared to be aged piperacillin on IgM > aged piperacillin on IgG > fresh piperacillin on IgM > aged piperacillin on IgA > fresh piperacillin on IgG > fresh piperacillin on IgA (Fig 1).

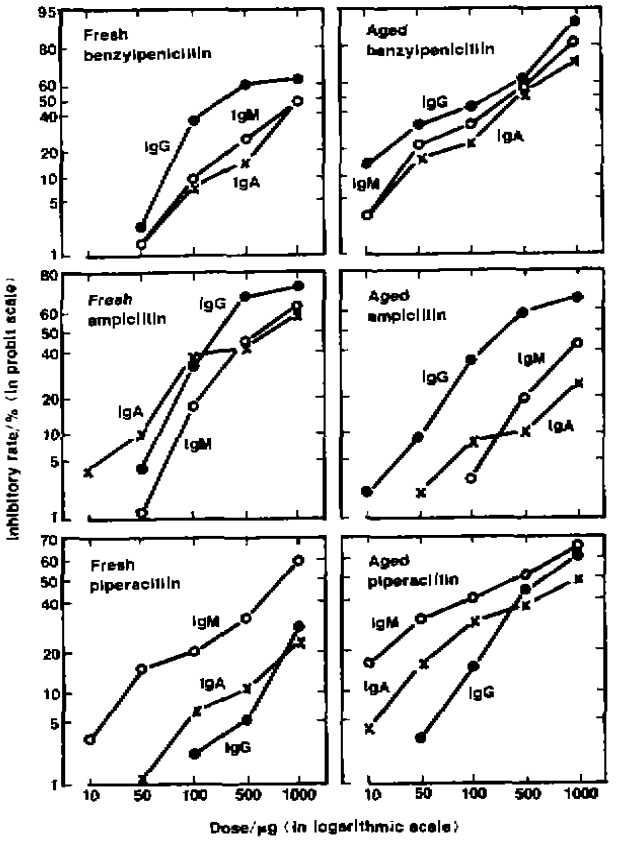

Fig 1. Inhibitory effect of penicillins on specific non-IgE antibodies in hapten inhibition assay.

DISCUSSION

Two major mechanisms responsible for penicillins allergy have been proposed[2,5,8,9]. One is fundamental reactivity of drugs to form penicilloylation of autologous proteins in the body, the other is the presence of the intrinsically antigenic impurities in the pharmaceutical preparations. Supporting those two mechanisms, the present study showed that specific non-IgE antibody response could be induced in mice *in vivo* immunized with freshly prepared benzylpenicillin solution containing very minute amounts of 15 kDa protein of *C jejuni* as impurity. The isotypes were mainly IgM and then IgG and IgA, which was consistent with our previous findings[6]. The findings reported here clearly demonstrated our hypothesis that freshly prepared benzylpenicillin or aged benzylpenicillin could induce different antibody responses which were previously found in human[7] and in mice[6]. Fig 1 showed that some parts of specific antibodies recognized benzylpenicillin molecule itself although

major parts of antibodies combined with the degraded products, polymer or transformation products, because aged benzylpenicillin showed higher inhibition to IgM, IgG and IgA than freshly prepared benzylpenicillin when the content was 10 – 1000 μg. This result was congruous with our previous results in children who were clinically suspicious of penicillins allergy[7], and was different from our other study in mice immunized with aged benzylpenicillin[6]. Moreover, we could draw out some other conclusions. First, benzylpenicillin showed higher inhibitory activity to IgG than to IgM and IgA, suggesting the avidity of IgG to benzylpenicillin might exceed IgM and IgA, which was contrary to the result in mice induced by aged benzylpenicillin in which the avidity of benzylpenicillin-reactive IgM exceeded IgG. Second, the antigenic activity of fresh benzylpenicillin was lower than that of aged benzylpenicillin, because only 50 % of mice in present study produced antibodies induced by fresh benzylpenicillin while 100 % of mice produced antibodies induced by aged benzylpenicillin in previous study[6]. This result was consonant with other studies that benzylpenicillin was unstable in solution and degraded to products which were more immunogenic and chemically reactive towards proteins than the drug itself[5]. Third, specific IgA was easily induced by fresh benzylpenicillin than aged benzylpenicillin on d 10 after immunization.

Owing to similarities in chemical structure, allergic reactions arising from β-lactam antibiotics were very likely to involve cross-allergenicity, and consequently the secondary choice of antibiotics for β-lactam hypersensitivity patients constituted a serious problem. The cross-reactivity of β-lactam drugs in allergic reactions with reference to IgE has been the subject of numerous studies. However, as the authors pointed out in previous reports[6,7], there has been little information concerning the role of non-IgE antibodies in immune cross reactivity. Having investigated the specific non-IgE antibody responses induced by freshly prepared benzylpenicillin, we further found that there were strong immune cross reactivities by non-IgE among benzylpenicillin, ampicillin and piperacillin. For cross reactivity between benzylpenicillin and ampicillin, the isotypes were mainly IgG, then IgM and IgA. For cross reactivity between benzylpenicillin and piperacillin, the isotypes were mainly IgM, then IgG and IgA.

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新鲜与陈旧青霉素诱导小鼠体内非 IgE 抗体应答的对比

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关键词 青霉素; 氨苄西林; 哌拉西林; 免疫球蛋白 G; 免疫球蛋白 M; 免疫球蛋白 A; 酶联免疫吸附试验; 半抗原; 交叉反应; 近交 BALB c 小鼠

目的: 研究新鲜青霉素和陈旧青霉素是否能诱导不同的非 IgE 抗体应答. **方法**: 酶联免疫吸附试验测定抗体应答, 半抗原抑制试验检测被抗体识别的抗原分子和抗原性交叉反应. **结果**: 新鲜青霉素免疫后诱导的特异性非 IgE 抗体类型主要是 IgM, 其次是 IgG 和 IgA. 部分抗体识别青霉素分子本身, 而大部分抗体结合降解产物或转化产物. 青霉素和氨苄西林间交叉反应性抗体主要是 IgG, 青霉素和哌拉西林间交叉反应性抗体则主要是 IgM. **结论**: 新鲜制备的青霉素与陈旧青霉素能诱导不同的非 IgE 抗体应答.