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尖吻蝮蛇毒和蝮蛇毒凝血酶样酶促凝的协同作用

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关键词 尖吻蝮蛇；蝮蛇；蛇毒；凝血酶类；血液凝固

目的：尖吻蝮蛇(*DA*)和蝮蛇(*AH*)蛇毒凝血酶样酶(TLE)对促凝作用的协同效果 **方法**：柱层析分离纯化 TLE 并进行体外凝血试验. **结果**：一个凝血单位 DATLE 为 2 7 μg，AHTLE 为 304 4 μg 它们单独作用形成的凝块很松脆，合用时凝血时间缩短，凝块的尿素溶解性下降. 再配伍阈下浓度的凝血酶或生理浓度的 Ca^{2+}，凝血时间进一步缩短，凝块能够回缩，不溶于尿素 5 mol·L^{-1}，对纤溶酶降解的抵抗性也增强. **结论**：DATLE 和 AHTLE 具有协同促凝作用，两者合用时凝血活性增强，凝块质量提高，可望成为一种有效的局部止血药.

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Benzylpenicillin induced specific non-IgE antibody response in mice

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KEY WORDS penicillins; IgM; IgG; IgA; enzyme-linked immunosorbent assay; haptens; cross reactions; drug hypersensitivity

AIM: To study whether or not the specific non-IgE antibody response in mice can be induced by benzylpenicillin *in vivo*. **METHODS**: Antibody response and antigenic cross reactions were determined by enzyme-linked immunosorbent assay (ELISA). Antigen molecules recognized by antibodies were tested by hapten inhibition assay. **RESULTS**: During d 1 - d 50 after immunization, positive % of specific IgM, IgG, and IgA to benzylpenicillin were 100 %, 50 % - 100 %, and 17 % - 100 %, respectively. IgM and IgG to benzylpenicillin also recognized ampicillin and piperacillin. The positive % of IgM and IgG to ampicillin were 23 % - 100 % and 50 % - 100 %, to piperacillin 43 % - 100 % and 50 % - 100 %, respectively. Aged benzylpenicillin showed an inhibitory effect on specific antibodies in a dose-dependent manner, and inhibitory % of specific IgM and IgG were 29 % - 87 % and 29 % - 71 %. However, freshly prepared benzylpenicillin had no effect. **CONCLUSION**: Specific non-IgE antibody response was successfully induced by benzylpenicillin in mice, in which the isotypes were mainly IgM and then IgG and IgA. Antibodies recognized degraded products, not benzylpenicillin molecule itself. Antigenic cross reactions occurred between benzylpenicillin, ampicillin, and piperacillin. Isotypes of antibodies responsible for cross reactions were mainly IgG and then IgM.

Many adverse drug reactions (ADR) are described as immunological mechanisms[1-3]. A large proportion of ADR to penicillin is mediated by the immune system, in which penicillin-reactive IgE antibody is considered to be responsible for acute

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allergic reactions[4-6]. However, in a number of cases, while symptoms suggested an anaphylactic reaction, *in vivo* and *in vitro* studies failed to show the presence of specific IgE antibody. At present, little is known about the role of non-IgE antibodies such as IgG, IgM, and IgA against penicillins in ADR. Our previous studies have successfully developed a sensitive enzyme-linked immunosorbent assay (ELISA) for detection of IgG against penicillins and found specific IgG in 8 of 13 subjects who were clinically suspicious of penicillins allergy[7]. The object of this study was to investigate whether or not the specific non-IgE antibody response in mice could be induced by immunization with benzylpenicillin.

MATERIALS AND METHODS

Reagents Bovine serum albumin (BSA) was obtained from Sigma Chemical Co (USA). Horseradish peroxidase-labelled sheep anti-mice IgG, IgM, and IgA were from Gibco BRL (USA). Protein 15 kDa of *Campylobacter jejuni* was from Shanghai Institute of Immunology. Polystyrene microtiter plates (96-well) were from Coster (USA). Benzylpenicillin, ampicillin, and piperacillin were obtained from Shanghai Third Pharmaceutical Factory.

Mice and immunization BALB c mice (♀ and aged 8 wk), weighing 20 ± 2 g, were from the Department of Experimental Animals, Shanghai Medical University. They were divided into 2 groups: experimental group ($n = 30$) and control ($n = 8$). Benzylpenicillin was dissolved in normal saline and protein 15 kDa of *Campylobacter jejuni* was added into solution to 5 mg·L^{-1}. The benzylpenicillin solution was stored at 20 −22 ℃ for 24 h. The mice in experimental group were immunized with ip benzylpenicillin 9 kU (0.2 mL/mouse) from d 1 to d 6 and from d 15 to d 17, while the mice in control group were injected ip normal saline. Mice were bled on d 10, d 20, d 35, d 50, and serum samples were stored at −20 ℃.

ELISA for detection of antibodies The ELISA was performed[7] with modification. Briefly, benzylpenicillin-BSA, ampicillin-BSA or piperacillin-BSA conjugates were coated on microtiter plates. Prediluted sera (1 : 50) were added to wells in triplicate and incubated at 37 ℃ for 2 h. Horseradish peroxidase-labelled sheep anti-mice IgG, IgM or IgA were added to wells and incubated. After washing, the substrate solution was added to wells and incubated at 37 ℃ for 0.5 h. The absorbance (A) was read at 492 nm using EIA Reading (Bio-Rad, USA). The mean A value and standard deviation of control sera were calculated as threshold ($t = \bar{x} + 3\ s$), above which the tested serum was considered as specific antibody positive. The level of antibody was expressed as enzyme index.

Hapten inhibition assay The assay was performed[7] with modification. Briefly, 50 μL of a prediluted mixed serum (1 : 25) from experimental mice on d 35 after immunization with benzylpenicillin was preincubated with 50 μL of fresh or aged benzylpenicillin solution which had been stored at 37 ℃ for 6 h and at 4 ℃ overnight. ELISA was then performed.

RESULTS

Antibody response to benzylpenicillin In mice injected with benzylpenicillin, serum IgM and IgG against benzylpenicillin appeared rapidly on d 10 and then declined gradually, and were still detectable on d 50. The level of specific IgM was higher than that of IgG. The level of IgA against benzylpenicillin remained undetectable or very low until d 35 and then higher than those of IgM and IgG on d 50 (Tab 1, Fig 1).

Hapten inhibition response Aged benzylpenicillin showed an inhibitory effect in a concentration-

Tab 1. Positive % of serum antibodies against benzylpenicillin, ampicillin, and piperacillin in mice immunized with benzylpenicillin. $n = 30$.

Days after immunization	Number and positve % of mice d 10	d 20	d 35	d 50
IgM to benzylpenicillin	30 (100 %)	30 (100 %)	30 (100 %)	30 (100 %)
IgG to benzylpenicillin	30 (100 %)	30 (100 %)	15 (50 %)	23 (77 %)
IgA to benzylpenicillin	10 (33 %)	5 (17 %)	10 (33 %)	30 (100 %)
IgM to ampicillin	20 (67 %)	30 (100 %)	15 (50 %)	7 (23 %)
IgG to ampicillin	30 (100 %)	30 (100 %)	15 (50 %)	23 (77 %)
IgM to piperacillin	30 (100 %)	30 (100 %)	13 (43 %)	13 (43 %)
IgG to piperacillin	30 (100 %)	30 (100 %)	15 (50 %)	23 (77 %)

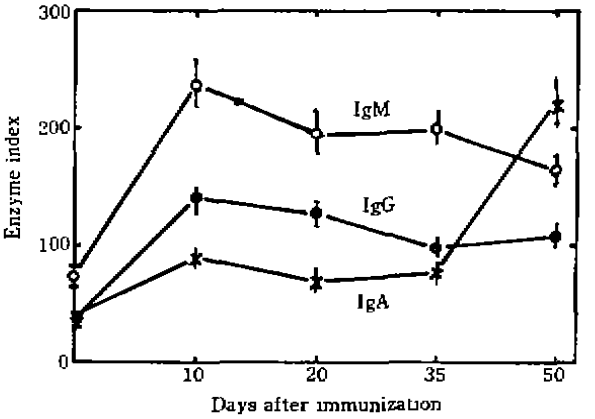

Fig 1. Kinetics of IgM, IgG, and IgA antibodies against benzylpenicillin in mice. $n = 30$ **mice,** $\bar{x} \pm s$.

dependent manner, in which the inhibitions of specific IgM and IgG were 29 % − 87 % and 29 % − 71 % when benzylpenicillin concentrations were 25 − 200 μg. Aged benzylpenicillin showed more inhibition to IgM than to IgG. But freshly prepared benzylpenicillin had barely inhibitory effect (Fig 2).

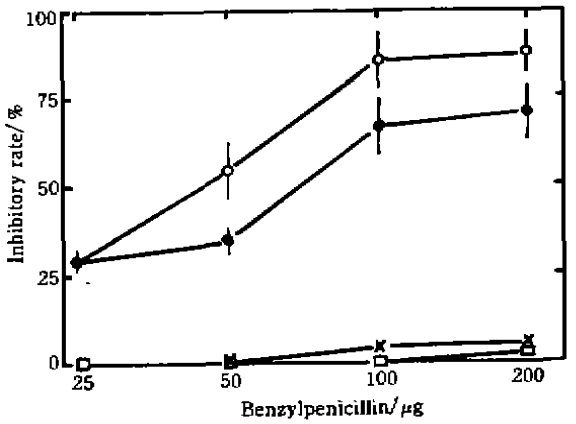

Fig 2. Inhibition of aged benzylpenicillin on IgM (○) and IgG (●); inhibition of fresh benzylpenicillin on IgM (×) and IgG (□). $n = 6$ **mice,** $\bar{x} \pm s$.

Antigenic cross reactions IgG and IgM from mice immunized with benzylpenicillin recognized not only benzylpenicillin but also ampicillin and piperacillin although the positive percentages of IgG and IgM to ampicillin and piperacillin were lower than those to benzylpenicillin. Positive percentages of IgG to ampicillin or piperacillin were higher than those of IgM (Tab 1).

DISCUSSION

According to the hapten hypothesis[1-3,6], it is believed that for a drug to generate an immunogen *in vivo* it must become covalently bound to an autologous carrier macromolecule, thereby forming a hapten and converting the carrier from an immunologically tolerated macromolecule to one recognized as foreign by the immune system. Supporting the hapten hypothesis, the present study demonstrated that benzylpenicillin induced strong antibody response. We adopted effective measure to enhance the antigenicity of benzylpenicillin: 1) mice were repeatedly immunized with aged benzylpenicillin, because other studies have shown that benzylpenicillin was unstable in solution and degradated to products which were more immunogenic and chemically reactive towards proteins than the drug itself[6]; 2) very minute amounts of 15 kDa protein of *Campylobacter jejuni* as impurity was added into benzylpenicillin solution, other researchers also found that removal of macromolecular impurities from therapeutic antibiotics brought striking reduction in the incidence of positive skin tests or PCA-eliciting activities[6,8]. The reason that the protein impurity increased the antigenicity of benzylpenicillin might be that impurity acted as carrier to activate T-cells which was necessary for B-cells to produce antibodies against benzylpenicillin. We noted that the levels of IgM and IgG to benzylpenicillin were lower on d 20 than on d 10, which was due to the blocking effect of secondly immunized benzylpenicillin on specific antibodies.

Hapten inhibition results clearly indicated that sera antibodies from mice recognized degraded products, polymer or transformation products, not benzylpenicillin molecule itself, because aged benzylpenicillin showed highly inhibitory activity while freshly prepared benzylpenicillin did not display effect. These results differed greatly from our previous studies in children in which at least some of IgG antibodies combined the benzylpenicillin molecule[7]. The most plausible explanation for the difference was that children were injected with

freshly prepared benzylpenicillin while mice were immunized with aged benzylpenicillin. In addition, the aged benzylpenicillin showed highly inhibitory percentages to IgM than to IgG, suggesting that the avidity of IgM to benzylpenicillin might exceed IgG.

The molecular nucleus of benzylpenicillin is 6-aminopenicillanic acid consisting of a β-lactam ring and a thiazolidine ring. Because this nucleus is present in all semisynthetic penicillins, it was not surprising that strong immunologic cross reactivity existed among benzylpenicillin, ampicillin or piperacillin, albeit to various degrees. However, positive percentages of IgM and IgG to ampicillin and piperacillin were lower than those to benzylpenicillin, suggesting that a portion of antibodies induced by benzylpenicillin had crossreactivity and others specific to benzylpenicillin. The results therefore reinforced recent findings[9-11] that the side chain of penicillins was an important determinant and that the side chain specific beta-lactam allergy should be paid more attention.

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青霉素诱导小鼠产生特异性非 IgE 抗体应答

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关键词 青霉素类；免疫球蛋白 M；免疫球蛋白 G；免疫球蛋白 A；酶联免疫吸附试验；半抗原；交叉反应；药物过敏 ELISA，抗体

目的：研究青霉素能否诱导小鼠体内特异性非 IgE 抗体应答 **方法**：酶联免疫吸附试验测定抗体应答和抗原性交叉反应，半抗原抑制试验检测被抗体识别的抗原分子 **结果**：青霉素免疫后的 50 天内，特异性 IgM、IgG、IgA 的阳性率为 100 %，50 % - 100 %，17 % - 100 % 特异性 IgM 和 IgG 还能识别氨苄西林和哌拉西林。陈旧青霉素以剂量依赖方式特异性阻断抗体结合，而新鲜青霉素无作用 **结论**：青霉素能在小鼠体内诱导特异性非 IgE 抗体应答，主要的抗体类型是 IgM，其次是 IgG 和 IgA。特异性抗体识别降解产物而非青霉素分子本身。青霉素、氨苄西林、哌拉西林三者间存在抗原交叉反应，参与交叉反应的抗体主要是 IgG，其次是 IgM

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