Immunology of the Monobactam Aztreonam

N. FRANKLIN ADKINSON, JR.,* EDWARD A. SWABB, and A. ARTHUR SUGERMAN

The Johns Hopkins School of Medicine at the Good Samaritan Hospital, Baltimore, Maryland 21239; The Squibb Institute for Medical Research, Princeton, New Jersey 08540; and The Medical Center at Princeton, Princeton, New Jersey 08540

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To assess the immunological cross-reactivity of the monobactam antibiotic aztreonam (AZ), rabbits were immunized with protein conjugates of benzylpenicillin, cephalothin (CEPH), and AZ. The resulting anti-benzylpenicilloyl (BPO) and anti-CEPH rabbit antibodies showed negligible cross-reactivity with AZ conjugated to human serum albumin (AZ-HSA), whereas anti-AZ showed negligible cross-reactivity with BPO-HSA and CEPH-HSA. Unlike benzylpenicillin and CEPH, unconjugated AZ was as effective as AZ conjugated to epsilon aminocaproic acid (AZ-EACA) in inhibiting the binding of homologous antibody. Studies with various analogs of AZ confirmed that immunoglobulin G (IgG) anti-AZ was entirely side-chain specific. The inhibition of the binding of human IgE anti-penicilloyl to BPO-HSA was studied in the presence of AZ-EACA, BPO-formyl lysine, and CEPH-EACA. Whereas CEPH-EACA displayed 3% cross-reactivity with BPO-lysine, AZ-EACA showed little or no cross-reactivity (<<0.9%). To assess the immunogenicity of AZ in humans, IgE and IgG antibodies were measured in sera from 36 healthy male volunteers receiving 0.5 or 1 g intravenously or intramuscularly every 8 h for 7 days. None of the subjects had detectable preexisting IgE reactive with AZ or demonstrated an IgE response to antibiotic administration. Four subjects gave evidence for naturally occurring IgG cross-reactive with AZ, but only one subject demonstrated a rise in IgG levels after exposure to AZ. This anti-AZ IgG did not cross-react with BPO or CEPH conjugates of bovine thyroglobulin and was completely side-chain specific. These studies suggest that AZ displays very low immunological cross-reactivity with other β-lactam antibiotics and may be only weakly immunogenic in humans.

Penicillin and cephalosporin antibiotics have consistently proven to be immunologically cross-reactive to varying degrees because of similar nuclear configurations despite marked differences in side-chain composition (2, 3, 6). This cross-reactivity and the immunogenicity (ability to stimulate an immune response) of available penicillins have hampered the administration of these antibiotics. Aztreonam (AZ) (SQ 26,776; formerly aztreonam) is a member of a newly discovered category of β-lactam antibiotics, the monobactams, characteristic by a monocyclic (as opposed to bicyclic penicillins and cephalosporins) β-lactam ring structure (10). Unlike naturally occurring monobactams made by bacteria, AZ is completely synthetic. AZ has potent bactericidal activity against aerobic gram-negative bacteria (9) and has been used successfully in early clinical trials to treat serious infections due to gram-negative bacteria. Because of the therapeutic potential of AZ and the clinical importance of cross-resistance among β-lactam antibiotics, studies have been undertaken to explore the potential for immunological cross-reactivity among AZ, benzylpenicillin, and cephalothin (CEPH). In addition, the immunogenicity of AZ administered in multiple doses to healthy male volunteers was investigated.

MATERIALS AND METHODS

Reagents. The major antigenic determinant of penicillin, toward which the immune response against this antibiotic is largely directed, is the penicilloyl moiety. This determinant is formed by simple hydrolysis of the β-lactam ring, with formation of an amide linkage predominantly with epsilon amino groups of lysine residues in plasma- or membrane-bound proteins. In analogous fashion, the cephalosporyl determinant is the principal immunogen for cephalosporin antibiotics, though antibodies specific for some cephalosporin side chains have occasionally been observed (2, 4). The multivalent conjugation of β-lactam antibiotics to large-molecular-weight carrier molecules appears to be an absolute requirement for both immunogenicity (ability to stimulate an immune response) and allergenicity (ability to elicit an immunologically mediated adverse effect). Hydrolysis of β-lactam antibiotics to produce covalent protein linkages occurs at measurable rates under physiological conditions. This is equally true for AZ, which becomes covalently bound to human serum protein (36 to 39% in 6 h, 68 to 70% in 24 h) at pH 7.4 in vitro (unpublished data, S. J. Lan, The Squibb Institute for Medical Research, Princeton, N. J.). For these reasons, we decided to prepare monovalent and polyvalent conjugates of AZ for use in cross-inhibition studies with analogous reagents prepared from benzylpenicillin and CEPH. The following reagents were prepared and characterized by The Squibb Institute for Medical Research by methods analogous to those previously developed for penicillin (5, 7); as immunogens, AZ36-57-segment, bovine thyroglobulin (BTG), CEPH11-92-BTG, and benzylpenicillin1-96-BTG; as test antigens, AZ-epsilon aminocaproic acid (EACA), CEPH-EACA, benzylpenicilloyl(BPO)-formyl lysine, AZ20-25 human serum albumin (HSA), CEPH50-HSA, BPO22-HSA, and AZ50-poly-l-lysine.

RAST assay. The radioallergosorbent (RAST) assay employed was an agarose-based (solid phase) immunoradiometric assay developed and standardized for the measurement of drug-specific rabbit antibodies. Conjugates of AZ-HSA were covalently bound to agarose beads by a cyanogen bromide technique. The immunoabsorbent had been previously standardized with rabbit antisera and was found to be acceptable in terms of specificity and low nonspecific bind-
After Staphylococcus protein immunoreactivity procedure, AZ buffer. When control (positive standards were run to remove naturally occurring antigens). Positive standards were run to remove naturally occurring antigens. The sera were evaluated for antibody by a modification of the RAST assay in which 125I-labeled Staphylococcus protein A was substituted as a detection protein in the second incubation period. Allergosorbs were prepared with drugs conjugated to a heterologous carrier, HSA, so that only the drug-specific immune response was detected. Sera from each of the three rabbits in each group were titrated to comparable levels of antibody binding. Inhibition studies were then performed with homologous and heterologous ligands.

**Antibody studies in humans receiving multiple doses of AZ.** The 36 healthy male subjects were 18 to 35 (mean, 22) years of age and were participating in a multiple-dose pharmacokinetic and safety study of AZ (8). None of them had been previously exposed to AZ. All subjects gave informed consent before entry into this study. The protocol for the study was approved by the Institutional Review Board of The Medical Center at Princeton.

The following AZ regimens were administered to nine subjects each: 500 mg intravenously every 8 h for 3 days, 1000 mg intravenously every 8 h for 3 days, 500 mg intramuscularly, q8h for 3 days, and 1000 mg intramuscularly, q8h for 3 days. Each regimen was continued for 7 days, with a last dose on the morning of day 8. Serum for measurement of possible antibody against AZ, by the RAST and radioimmunoprecipitation assays, was obtained from each volunteer at the following times: control (just before the first dose); 168 h (just before the last dose on the morning of day 8); day 14 (6 days after the last dose); and day 21 (13 days after the last dose).

**RESULTS**

Cross-reactivity studies with rabbit antisera. The results of studies of the cross-reactivity of antibodies raised in rabbits against the three β-lactam drug-protein conjugates are shown in Fig. 1. Figure 1a displays the mean (±standard error of the mean) inhibition for the three rabbits immunized with BPO-BTG. Cross-reactivity could be quantitated as the ratio, 100 × ID_{50} of the homologous conjugate, e.g., BPO-HSA/ID_{50} of a different, potentially cross-reacting β-lactam conjugate, e.g., CEPH-HSA, where ID_{50} is the amount of inhibitor required for 40% inhibition of antibody binding.

Note the consistent degree of cross-reactivity among the three rabbit antipenicillloyl antisera for CEPH-HSA (≈0.1%), as indicated by the small standard errors for data comparing the CEPH-HSA curve. In the case of the AZ-HSA conjugate, the cross-reactivity was consistently very weak (≈0.001%).
This observation suggested that the AZ-specific antibodies were largely, if not exclusively, side-chain specific. This fact was confirmed in a subsequent set of experiments employing several β-lactam compounds chemically similar to AZ, including ceftazidime (GR20263), a cephalosporin with a side chain identical to that of AZ. This aminothiazolyl cephalosporin produced complete inhibition of anti-AZ binding, whereas other monobactam antibiotics with differing side chains were essentially non-cross-reactive (<0.01%; data not shown).

The serial bleedings from all AZ-immunized rabbits were examined in detail. In each case, antibody binding was found to be totally inhabitable by free drug. This suggested that even late in the course of immunization, the predominant, if not exclusive, specificity of anti-AZ antibody was for the aminothiazolyl side chain. No antibody specific for the monobactam nucleus could be demonstrated in these rabbit studies.

Cross-reactivity of human IgE anti-penicilloyl antibodies. Serum samples were obtained from three penicillin-allergic human subjects having positive IgE-dependent skin tests to BPO-polylysine. Suitable dilutions were chosen to give comparable levels of binding of IgE anti-penicilloyl antibody in the RAST assay. Inhibition studies were performed with homologous and heterologous polyvalent and monovalent drug conjugates as well as the unconjugated drug. Results with all three sera were comparable and are summarized in Table 1. A representative inhibition study is shown in Fig. 3.

Human IgE anti-penicilloyl antibody was very weakly cross-reactive (<0.001 to <0.9%) with AZ conjugates (Table 1). As expected, CEPH conjugates were cross-reactive at higher levels from 0.04 to 3%. Similar results were obtained with inhibition studies by using three sera with high titers of IgG anti-penicilloyl antibody in the radioimmuno precipitation assay (Table 1).

Primary immunogenicity of AZ in humans. Results of the RAST and radioimmunoprecipitation assays revealed that naturally occurring IgE cross-reactive with AZ was absent from the predose (control) sera of all study subjects, and there was no IgE response after the parenteral administration of AZ. In contrast, by using the radioimmunoprecipitation assay, predose sera from 4 of 36 subjects displayed small amounts of AZ-inhibitable binding activity, which may

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**FIG. 1.** Cross-reactivity of rabbit antibodies raised against BTG conjugates of three β-lactam compounds, benzylpenicillin (a), CEPH (b), and AZ (c). The inhibitors used were polyvalent conjugates of BPO-HSA, CEPH-HSA, and AZ-HSA. (b) E1, E2, and E3 refer to data from three individual rabbits. Bars indicate standard errors.

Figure 1b shows the results obtained with rabbit anti-cephalosporin antisera. Although the cross-reactivity with the penicilloyl specificity was variable (individual rabbit responses are shown as E1, E2, and E3), cross-reactivity with the AZ conjugate was consistently negligible.

Figure 1c indicates the results with rabbit anti-AZ antiserum. Cross-reactivity with the penicillin and cephalosporin conjugates tested was minimal (<0.001%).

Finally, it was observed that free AZ was equally effective on a molar basis with AZ-EACA in inhibiting anti-AZ antibody binding to the AZ-HSA immunoabsorbent (Fig. 2).

**FIG. 2.** Cross-reactivity of rabbit antibody raised against a BTG conjugate of AZ. The monovalent inhibitors used were free AZ, free CEPH, free benzylpenicillin (BP), CEPH-EACA, and BPO conjugated to formylated lysine (BPO-FLYS).
TABLE 1. Cross-reactivity of anti-penicilloxy antibodies obtained from sera of penicillin-allergic human subjects

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IgE anti-BPO</th>
<th>IgG anti-BPO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I_{D_{50}}$ (moles added)*</td>
<td>Relative efficiency*</td>
</tr>
<tr>
<td>BPO$_{22}$-HSA</td>
<td>$3 \times 10^{-12}$</td>
<td>1</td>
</tr>
<tr>
<td>CEPH$_{30}$-HSA</td>
<td>$8 \times 10^{-9}$</td>
<td>$4 \times 10^{-4}$</td>
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<tr>
<td>AZ$_{30}$-HSA</td>
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<td>$&lt;&lt;10^{-5}$</td>
</tr>
<tr>
<td>BPO-FLYS$^c$</td>
<td>$9 \times 10^{-9}$</td>
<td>$3.3 \times 10^{-4}$</td>
</tr>
<tr>
<td>CEPH-EACA</td>
<td>$3 \times 10^{-7}$</td>
<td>$1 \times 10^{-5}$</td>
</tr>
<tr>
<td>AZ-EACA</td>
<td>$&gt;&gt;1 \times 10^{-6}$</td>
<td>$3 \times 10^{-6}$</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>$8 \times 10^{-7}$</td>
<td>$3.8 \times 10^{-6}$</td>
</tr>
<tr>
<td>CEPH</td>
<td>$6 \times 10^{-6}$</td>
<td>$5 \times 10^{-7}$</td>
</tr>
<tr>
<td>AZ</td>
<td>$(2 \times 10^{-5})^d$</td>
<td>$1.5 \times 10^{-7}$</td>
</tr>
</tbody>
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* $I_{D_{50}}$, Moles of inhibitor required for 40% inhibition of antibody binding.
* Relative efficiency of inhibition of binding to IgE anti-BPO is the ratio, $I_{D_{50}}$ of BPO$_{40}$-HSA/$I_{D_{50}}$ of inhibitor; defined as unity for BPO$_{40}$-HSA.
* Cross-reactivity is defined separately for each group of three inhibitors as the ratio, $100 \times I_{D_{50}}$ of the BPO conjugate or benzylpenicillin/$I_{D_{50}}$ of the CEPH or AZ conjugate or free compound; defined as 100% for BPO$_{40}$-HSA, BPO-formyl lysine, and benzylpenicillin.
* $>>$, No significant inhibition was observed at the highest concentration tested.
* FLYS, Formyl lysine.
* $I_{D_{50}}$ was estimated by extrapolation.

have represented low levels of naturally occurring IgG that was cross-reactive for AZ. This preexisting cross-reacting antibody was boosted by AZ exposure in only one of the four subjects. The counts per minute of $^{125}$-AZ-HSA that was bound by the pretreatment sera and inhibitable by AZ-BTG was 148 cpm greater than negative control sera, which averaged 547 ± 26 cpm (mean ± standard error of the mean, $n = 4$). This net inhibitable binding was unchanged at 168 h but rose to 312 cpm above control at day 14 and to 974 cpm above background at day 21. The anti-AZ IgG was not cross-reactive with BPO-BTG or CEPH-BTG. However, free AZ and AZ-BTG were equally effective as inhibitors. Dialysis of sera collected from human subjects did not affect the observed result, indicating that free AZ or metabolites did not interfere with the assay.

DISCUSSION

These immunochemical studies suggest that AZ is a β-lactam antibiotic which may not be significantly cross-reactive with anti-penicillin and anti-cephalosporin antibodies. Inhibition studies with both prepared rabbit antisera and human sera from penicillin-allergic patients indicate that anti-penicillloy antibodies bind poorly, if at all, with AZ and its conjugates. The expected cross-reactivity with CEPH was demonstrated, indicating that the experimental system is capable of identifying partial cross-reactivity. Based on these limited studies, it is reasonable to anticipate that at least those penicillin-allergic patients with a major determinant (penicillloy) allergy may receive treatment with monobactam antibiotics with little likelihood of cross-allergenicity. This possibility is very attractive clinically, because penicillin and cephalosporin hypersensitivity generally contraindicate the use of all currently available β-lactam antibiotics.

In rabbits immunized with AZ-protein conjugates and in the single human anti-AZ antisera studied to date, the principal antibody specificity observed is for the side chain of AZ. No antibodies recognizing the monobactam nucleus could be demonstrated. This interesting finding suggests that various monobactam antibiotics may not be highly cross-reactive with each other if side chains differ significantly. Therefore, any allergic sensitivity induced by a monobactam antibiotic may preclude the future use only of the sensitizing drug and not necessarily the family of monobactams as a whole. In addition, the predominant side-chain specificity of AZ antibodies may allow the free (unconjugated) drug to provide significant inhibition in vivo of any potential immunopathological reaction which might otherwise occur in some patients who produce drug-specific antibodies. Theoretically, this feature of the drug-specific immune response further reduces the likelihood of drug allergy among patients receiving multiple courses of therapy.

No IgE antibody response was detectable in 36 subjects receiving multiple doses of AZ, and only 1 of 36 subjects demonstrated an IgG response. These apparent incidences of IgE and IgG response to AZ, 0 and 3%, respectively, were lower than the 11% IgE and 60% IgG response to penicillin that has been observed in hospitalized patients receiving penicillin therapy (1). However, the results of the present study do not conclusively establish a reduced immunogenicity of AZ relative to other β-lactam compounds, in the absence of concurrent evaluation of penicillin and cephalosporin treatment in comparable subjects.

![FIG. 3. Cross-reactivity of human IgE-anti-BPO antibody with polyvalent β-lactam conjugates of HSA. Antibiotics conjugated to HSA were BPO, CEPH, and AZ.](http://aac.asm.org/Downloaded from October 18, 2017 by guest)
The low incidence (0 to 3%) of a drug-specific antibody response among humans receiving full therapeutic doses of AZ suggests that AZ is only weakly immunogenic, compared with other β-lactam antibiotics. Ultimately, however, multiple courses of therapy with AZ will have to be examined to establish the absolute immunogenicity of AZ, because a secondary antibody response may not occur upon first exposure to the drug. Furthermore, it remains to be shown that the β-lactam hydrolysis product of AZ represents the immunodominant conformation of the drug. Other haptenic conformations of the drug may also prove to be immunogenic in some or all subjects receiving the antibiotic.

This study provides the first demonstration of a human IgG antibody response to parenterally administered AZ. The greatest rise in serum IgG levels occurred 2 weeks after the last AZ dose. It is noteworthy that this IgG antibody to AZ did not cross-react with the major penicillin or cephalosporin antigenic determinants, a confirmation of findings obtained with sera from AZ-immunized rabbits. Low levels of naturally occurring IgG cross-reactive with AZ were seen in 4 of 36 subjects not previously exposed to AZ, but these levels were generally not boosted by AZ exposure and are, therefore, likely to be insignificant with regard to the potential for mediating drug-induced immunopathological events. The origin of these naturally occurring AZ-binding antibodies remains unexplained.

If future studies confirm that AZ is poorly cross-reactive with other β-lactam antibiotics, AZ and similar monobactams may be of particular value for patients who have demonstrated sensitivity to penicillins or cephalosporins. If, in addition, its immunogenicity proves to be weak, the long search for a relatively non-allergenic β-lactam antibiotic may at last come to fruition. Clinical studies to evaluate these two possibilities further are now in progress.

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LITERATURE CITED


