Comparative Capacity of Orally Administered Amoxicillin and Parenterally Administered Penicillin-Streptomycin to Protect Rabbits against Experimentally Induced Streptococcal Endocarditis

RAMON PUJADAS,1 ENRIQUE ESCRIVA,1 JAVIER JANE,1 FRANCISCO FERNANDEZ,1 PILAR FAVA,2 AND JAVIER GARAU3††

Department of Internal Medicine1 and Service of Microbiology,2 Hospital Central Q. S. “La Alianza,” Barcelona, and Infectious Diseases Unit, Hospital “Príncipes de España,” University of Barcelona, Barcelona,3 Spain

Received 19 July 1985/Accepted 25 February 1986

A single-intramuscular-dose immunization regimen with a penicillin G-streptomycin combination was compared with three oral-dose amoxicillin regimens for the capacity to prevent Streptococcus sanguis infections of experimentally induced valvarular heart lesions in rabbits. Challenge doses of 10⁵, 10⁶, and 10⁸ CFU of a strain of S. sanguis equally susceptible to penicillin and amoxicillin were used in this study. Measured by recovery of test organisms from endocardial lesions, the lowest concentration of these inocula was infective for 60% of the recipients; the two higher-concentration inocula were infective for all recipients. The penicillin G-streptomycin combination provided complete protection against infection with inocula of all sizes. A single-oral-dose amoxicillin regimen (50 mg/kg of body weight) prevented endocarditis when rabbits were challenged with 10⁶ CFU, but protection diminished with increasing inoculum concentrations. Similar results were achieved when five oral doses of amoxicillin (8.5 mg/kg of body weight) added at 8-h intervals were included in the single-oral-dose regimen. In contrast, when rabbits received two oral doses of amoxicillin (50 mg/kg of body weight) with a 10-h interval between doses, prophylaxis was fully effective with even the highest inoculum concentration.

Patients with valvular heart disease or intracardiac prostheses are presumed to be at risk for infective endocarditis (IE) after dental work. Experimental studies on the prevention of streptococcal endocarditis suggested that application of bactericidal antibiotics would reduce this risk and that a penicillin G-streptomycin combination was the most effective regimen (5, 10). This led the American Heart Association to suggest that patients at high risk should be given penicillin plus an aminoglycoside intramuscularly for prophylaxis of IE caused by viridans streptococci (1). In Europe, however, orally administered amoxicillin has been used for coverage because of the following reasons. Compliance with the American Heart Association regimens has been reported to be low (7), essentially all strains of viridans streptococci are susceptible to amoxicillin, and this antibiotic is well absorbed and well tolerated when administered orally (12, 13).

Since there are not many in vivo studies describing the efficacy of orally administered amoxicillin, we decided to determine the usefulness of single- and multiple-dose regimens of this agent in the rabbit endocarditis model, comparing such with the benefits of a single-intramuscular-dose penicillin G-streptomycin regimen. We report herein the results of these experiments on prophylaxis against experimental endocarditis caused by a strain of Streptococcus sanguis susceptible to penicillin G and amoxicillin.

MATERIALS AND METHODS

Infecting organism. A penicillin-sensitive strain of S. sanguis biotype II (S. sanguis HPE 93) obtained from a patient with subacute bacterial endocarditis was used throughout these studies. For stock purposes, 1-ml aliquots of an 18-h culture of this strain in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) enriched with 5% sheep erythrocytes were stored at −20°C. For each experiment, an aliquot was thawed, inoculated into 50 ml of the medium described above, and incubated for 18 h at 37°C.

In vitro susceptibility studies. The MICs and MBCs of penicillin G, streptomycin, and amoxicillin were measured by a standard twofold tube dilution method. An inoculum of approximately 10⁵ CFU of S. sanguis (0.05 ml of a 1:100 dilution of an overnight culture) was added to tubes containing serial twofold dilutions of antibiotic standards in 1 ml of Mueller-Hinton broth (Difco). The MIC was determined by visual inspection after 24 h of incubation at 37°C in a candle jar. The MBC was determined by spreading 0.1 ml from each clear tube onto the surface of sheep blood agar plates. Penicillinase (Difco) was added to the agar at a concentration of 50 U/ml. The plates were incubated at 37°C in a candle jar for 48 h. The MBC was read as the lowest concentration of antibiotic that killed 99.9% or more of the initial inoculum. This corresponded to 10 or fewer surviving colonies per plate. Each determination was done three times.

Time-kill studies. The effects of penicillin G, streptomycin, amoxicillin, and the penicillin-streptomycin combination on this strain of S. sanguis were measured by means of time-kill curves performed in Mueller-Hinton broth by a modification of the method of Wolfe and Johnson (14). Penicillin G was tested at concentrations of 1 and 10 μg/ml, streptomycin was tested at concentrations of 10 and 55 μg/ml, amoxicillin was tested at concentrations of 1 and 21 μg/ml, and the penicillin G-streptomycin combination was tested at concentrations of 0.5 and 5 μg/ml, respectively. A bacterial inoculum of 10⁶ CFU of S. sanguis (1 ml of a 1:200 dilution of an overnight culture) was added to tubes containing 1 ml of Mueller-Hinton broth with or without antibiotic as a growth control. All tubes were incubated in 10% CO₂ at
37°C. Before and after 6, 24, and 48 h of incubation, 0.1 ml volumes of either undiluted or serially diluted cultures were streaked onto sheep blood agar plates containing 50 U of penicillinase per ml which were then incubated for 48 h in 10% CO₂ at 37°C. When growth was absent, the time-kill study was repeated with plating every 2 h during the "presterilization" interval. Each experiment was done three times. The minimum sensitivity of this method was 10 CFU per ml of undiluted culture.

**Penicillin G, streptomycin, and amoxicillin levels in serum.**

To assist with selection of the doses to be used in the experimental animal studies, we determined the concentrations of penicillin G, streptomycin, and amoxicillin in the sera of human volunteers given the doses of these agents recommended for prophylaxis of streptococcal endocarditis. Twelve individuals (10 men and 2 women, from 21 to 36 years of age and 58 to 71 kg in body weight) in four subgroups of three participated in this study. Blood was drawn 1, 4, 8, 12, and 24 h after administration of an intramuscular dose of 1,000,000 U of crystalline penicillin G plus 600,000 U of procaine penicillin G. Likewise, blood was drawn 1, 4, 8, and 12 h after administration of an intramuscular dose of 1 g of streptomycin or an oral dose of 3 or 0.5 g of amoxicillin. Levels of each antibiotic in serum were determined separately by the agar well diffusion method of Bennett et al. (2), with Bacillus subtilis as the assay organism.

On the basis of results of the study described above, doses of 40,000 or 80,000 U of procaine penicillin G per kg of body weight administered intramuscularly with or without 25 mg of probenecid per kg of body weight, 15 or 20 mg of streptomycin per kg of body weight administered intramuscularly, and 50 or 8.5 mg of amoxicillin per kg of body weight administered by gavage with or without 25 or 10 mg of probenecid per kg of body weight were administered to groups of three rabbits. Blood was drawn from an ear vein of each rabbit 1, 4, 8, 12, and 24 h after dosage for measurement of concentrations of the respective agents in serum.

**Assessment of prophylactic activity.** Sterile vegetables were produced in 225 New Zealand White rabbits (3.0 to 3.7 kg in body weight) by the procedure of Perlman and Freedman (11) as modified by Durack and Beeson (4). Briefly, this procedure, carried out in the anesthetized animal, involves introducing a polyethylene catheter into the right carotid artery, advancing the catheter until it reaches the aortic valve, and then securing it in place for the duration of the experiment.

Seventy-two h after catheterization, groups of 6 to 10 rabbits were inoculated via the marginal ear vein with approximately 10⁵, 10⁶, or 10⁷ CFU of S. sanguis. In each of 30 sessions, one or two rabbits were randomly assigned to a control group, and the remainder were randomly assigned to a prophylactic regimen. Inocula were prepared from an 18-h culture by sedimenting the growth by centrifugation, washing the sediment three times with phosphate-buffered saline, resuspending it in phosphate-buffered saline, and quantifying numbers by optical density measurements to attain 10⁶ CFU per unit of volume. Inocula of 10⁵ and 10⁶ CFU were obtained by serial dilution in phosphate-buffered saline. The actual numbers of CFU injected were verified by the culture of 0.1-ml portions from serial 10-fold dilutions in blood agar pour plates.

One hour before challenge, subgroups of rabbits received one of the following regimens: (i) a single intramuscular dose of procaine penicillin G (80,000 U/kg of body weight) plus probenecid (25 mg/kg of body weight), together with streptomycin (20 mg/kg of body weight (regimen PCN plus SM); (ii) a single oral dose of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) (regimen AMOX-1); (iii) two oral doses of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) with a 10-h interval between doses (regimen AMOX-2); (iv) an oral dose of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) followed by five doses of amoxicillin (8.5 mg/kg of body weight) plus probenecid (10 mg/kg of body weight) with 8-h intervals between doses (regimen AMOX-6).

Rabbits were sacrificed by the intramuscular injection of pancuronium bromide 72 h after the last dose of antibiotic (or after inoculation in control groups). One milliliter of blood was drawn from the inferior vena cava and plated on blood agar. The hearts were removed aseptically, and the vegetations were excised, weighed, and homogenized in 1.0 ml of tryptic soy broth (Difco) in glass tissue grinders. Of this homogenate, 0.1 ml was used for serial dilutions. The remaining 0.9-ml and 0.1-ml portions of various dilutions were incorporated into blood agar plates containing 50 U of penicillinase per ml and incubated for 48 h at 37°C in a candle jar.
TABLE 1. Levels of antibiotics in serum from rabbits and human volunteers

<table>
<thead>
<tr>
<th>Time after a dose (h)</th>
<th>Rabbits</th>
<th>Human volunteers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Amoxicillin (50 mg/kg of body wt) + probenecid (25 mg/kg of body wt), orally</td>
<td>Amoxicillin (8.5 mg/kg of body wt) + probenecid (10 mg/kg of body wt), orally</td>
</tr>
<tr>
<td>1</td>
<td>21.0 ± 5.2</td>
<td>6.4 ± 1.7</td>
</tr>
<tr>
<td>4</td>
<td>7.1 ± 2.1</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>8</td>
<td>0.8 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>12</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>24</td>
<td>0.6 ± 0.2</td>
<td>0.0 ± 0.0</td>
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</tbody>
</table>

* Mean ± standard deviation of the levels in serum determined in at least three rabbits or three healthy human volunteers.

Protection against challenge with inocula of all sizes. The AMOX-1 regimen provided full protection against challenge with $10^6$ CFU but not against challenge with $10^8$ CFU. It should be noted, however, that the mean bacterial densities in vegetations of the amoxicillin-treated rabbits in which IE developed were significantly lower than in control rabbits challenged with the same inoculum ($P < 0.05$ for each inoculum). Thus, although not fully effective, amoxicillin reduced both the incidence and intensity of infection.

The AMOX-2 regimen provided complete protection against challenge with inocula of all sizes. The AMOX-6 regimen provided such protection only against a challenge with $10^4$ CFU and much less protection against inocula of $10^6$ and $10^8$ CFU, a level comparable to that achieved with a single oral dose of amoxicillin (50 mg/kg of body weight).

Likewise, mean bacterial densities in vegetations of rabbits treated with the AMOX-6 regimen in which IE developed were significantly lower than in control rabbits challenged with the same inoculum ($P < 0.05$ and $P < 0.025$ for inocula of $10^6$ and $10^8$ CFU, respectively).

All untreated animals with culture-positive vegetations also had positive blood cultures. In contrast, S. sanguis was recovered from the blood of only 11 out of 16 rabbits in which attempted prophylaxis with regimens AMOX-1 or AMOX-6 had failed.

DISCUSSION

In 1982, the British Society for Antimicrobial Chemotherapy recommended that amoxicillin be administered orally in a 3-g dose before dental procedures to prevent IE for patients with or without prosthetic heart valves (15). How-

![FIG. 1. Bactericidal activity of amoxicillin (AMOX), penicillin G (PCN), streptomycin (SM), and the penicillin G-streptomycin combination (PCN + SM) against S. sanguis. Each colony count represents the mean of three determinations.](http://aac.asm.org/)

TABLE 2. Results of prophylaxis with each regimen in rabbits challenged with a penicillin- and amoxicillin-susceptible strain of S. sanguis

<table>
<thead>
<tr>
<th>Prophylactic regimen</th>
<th>Inoculum (CFU)</th>
<th>No. of sterile vegetations/total vegetations</th>
<th>Mean (±SD) log$_{10}$ CFU/g of culture-positive vegetation</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$10^6$</td>
<td>6/15</td>
<td>6.71 ± 1.14</td>
</tr>
<tr>
<td>PCN + ST$^a$</td>
<td>$10^6$</td>
<td>0/15</td>
<td>7.56 ± 0.64</td>
</tr>
<tr>
<td>AMOX-1$^b$</td>
<td>$10^6$</td>
<td>15/15</td>
<td>7.91 ± 0.62</td>
</tr>
<tr>
<td>AMOX-2$^c$</td>
<td>$10^6$</td>
<td>15/15</td>
<td>4.32 ± 0.78</td>
</tr>
<tr>
<td>AMOX-6$^d$</td>
<td>$10^6$</td>
<td>15/15</td>
<td>3.41 ± 0.30</td>
</tr>
</tbody>
</table>

$^a$ PCN + ST, Single dose of procaine penicillin G (80,000 U/kg of body weight) plus streptomycin (20 mg/kg of body weight) and probenecid.

$^b$ AMOX-1, Single dose of amoxicillin (50 mg/kg of body weight) plus probenecid, orally.

$^c$ AMOX-2, Two doses of amoxicillin (50 mg/kg of body weight) plus probenecid, administered orally with a 10-h interval between doses.

$^d$ AMOX-6, Single dose of amoxicillin (50 mg/kg of body weight) plus probenecid and five subsequent doses of amoxicillin (8.5 mg/kg of body weight) plus probenecid, administered orally with 8-h intervals between doses.
ever, although this regimen seems promising, high-risk patients should be treated with the safest possible one, which seemed to be a penicillin G-streptomycin combination (1, 5, 10). Thus, our purpose was to compare the efficacy of various amoxicillin oral-dose regimens with that of the intramuscular penicillin G-streptomycin combination.

It is unlikely that successful prophylaxis against S. sanguis IE with a single oral dose of amoxicillin was achieved through bacterial killing, because it took 14 to 20 h for bactericidal concentrations to kill this strain in vitro. Furthermore, since protection provided by bacterial killing seems independent of inoculum concentration (6), the partial failure of the AMOX-1 regimen when rabbits were challenged with $10^6$ or $10^8$ CFU also suggests that protection was achieved by an alternative mechanism(s).

When we attempted to increase the protection achieved with the single-dose amoxicillin regimen by the administration of five subsequent low doses, the efficacy was not significantly improved. This could be related to limited antibiotic penetration into vegetation (9), achieving low inner concentrations after administration of oral doses of amoxicillin (8.5 mg/kg body weight). PCN-plus-SM and AMOX-2 regimens (which achieved bacterial killing in vitro) were significantly more effective than single-dose orally administered amoxicillin, protecting all rabbits against even the highest-concentration inoculum. Further work is needed to assess the relative merits of the PCN-plus-SM versus AMOX-2 regimens.

When lesions of AMOX-1- or AMOX-6-treated rabbits were culture positive, the numbers of organisms recovered were significantly lower than those in the lesions of untreated rabbits. Since inoculation of $10^7$ CFU of S. sanguis into rabbits leads to an immediate colonization of preexisting vegetation, achieving high bacterial densities in 24 h (4), the low counts found in infected amoxicillin-treated rabbits might have been related to a postantibiotic effect in vivo (3).

Our results appear to confirm recent findings (6) suggesting that amoxicillin can prevent IE in the absence of bacterial killing. Although intramuscular injection of penicillin G-streptomycin, a synergistic combination with high bactericidal activity (8), is probably the safest regimen to use, a single-high-concentration-dose amoxicillin regimen seems to protect acceptably well against day-to-day bacteremia after dental work; however, it failed manifestly against high inoculum concentrations of resistant strains (6) and, in our study, against high inoculum concentrations of this slowly killed susceptible strain of S. sanguis. Our findings suggest that lengthening the maintenance of bactericidal levels of amoxicillin in serum might enhance protection against some of these strains. Thus, the administration of a second high-concentration oral dose and the addition of probenecid could be wise alternatives specially indicated for high-risk patients or septic oral manipulations that could be associated with higher inoculum concentrations.

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LITERATURE CITED