Efficacy of Orally Administered Penicillin V for Prophylaxis of Experimentally Induced Streptococcal Endocarditis

RAMON PUJADAS,†* ENRIQUE ESCRIVA,† JAVIER JANE,† M. CARMEN GALERA,‡ PILAR FAVA,‡ JAVIER GARAU,‡ AND BEATRIZ MIRELIS‡

Department of Medicine† and Service of Microbiology,‡ Hospital Central Q.S. La Alianza, Department of Medicine, Hospital Mutua de Terrassa, Terrassa,§ and Service of Microbiology, Hospital de Sant Pau,¶ Barcelona 08017, Spain

Received 19 February 1987/Accepted 15 July 1987

Four oral penicillin V regimens were compared for the ability to prevent Streptococcus sanguis infection of experimentally induced valvular heart lesions in rabbits. Challenge doses of 10^4, 10^6, and 10^8 CFU of a penicillin-susceptible strain of S. sanguis were used in this study. Measured by recovery of test organisms from endocardial lesions, the lowest-concentration inoculum was infective for 53% of the recipients; the higher-concentration inocula were infective for all recipients. A single-oral-dose penicillin V regimen (36 mg/kg of body weight) prevented endocarditis when rabbits were challenged with 10^6 CFU, but protection diminished with increasing inoculum concentrations. In contrast, addition of a second penicillin V dose (18 mg/kg of body weight) administered with a 7-h interval between doses achieved fully effective prophylaxis against even the highest inoculum tested (10^8 CFU). A repeated set of experiments in which half the dose of penicillin V was administered showed significantly reduced protection against S. sanguis endocarditis.

Intramuscular injection of a synergistic combination of a β-lactam plus aminoglycoside is probably the safest therapy for preventing infective endocarditis (IE) following dental work in susceptible patients (9). However, most patients prefer to avoid injections and attend a dental practitioner who works outside the hospital, where there are often difficulties in arranging for parenteral prophylaxis. These facts clearly justify general use of oral prophylaxis in current practice.

Using the rabbit model of experimental endocarditis, we have recently shown that a single oral dose of amoxicillin (50 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) prevented IE in all animals challenged with 10^8 CFU of a strain of Streptococcus sanguis susceptible to amoxicillin (S. sanguis HPE93) and that addition of a second dose (50 mg/kg of body weight) 10 h later enhanced protection against higher inocula (11). However, a loading 2-g oral dose of penicillin V 1 h before dental work followed by a 1-g dose 6 h afterwards has been recommended by the American Heart Association (AHA) committee on prevention of bacterial endocarditis (13). Since there are no in vivo studies describing the efficacy of orally administered penicillin V, we decided to determine the efficacy of single- and two-dose penicillin regimens in the rabbit endocarditis model and to compare it with the benefits of amoxicillin. We report here the results of these experiments against experimentally induced endocarditis caused by S. sanguis HPE93, a strain equally susceptible to phenoxymethylpenicillin and amoxicillin in vitro, which has been used in previous studies (11).

MATERIALS AND METHODS

Infecting organism. A previously described (11) strain of S. sanguis biotype II (S. sanguis HPE93) from a patient with subacute bacterial endocarditis was used throughout the study. For stock purposes, 1-ml portions of an 18-h culture of this strain were kept in brain-heart infusion broth (Difco Laboratories, Detroit, Mich.) enriched with 5% sheep erythrocytes and stored at −20°C. For each experiment, a portion was thawed, inoculated into 50 ml of this medium, and incubated for 18 h at 37°C.

In vitro susceptibility studies. The MIC and MBC of penicillin V were measured by a standard twofold tube dilution method (11), the MBC being defined as the lowest concentration of antibiotic that killed 99.9% or more of the initial inoculum (10^5 CFU/ml).

Time-kill curves with 1 and 10 g of penicillin V per ml were performed in Mueller-Hinton broth by a modification of the method of Wolfe and Johnson (14) as previously described (11).

Penicillin V levels in serum. To select the dose to be used in the experimental-animal studies, we first determined the concentrations of penicillin V in the serum of human volunteers given the doses of this agent recommended for prophylaxis against streptococcal endocarditis by the AHA (13). Six individuals (five men and one woman, 21 to 33 years of age and weighing 60 to 78 kg) in two subgroups of three each participated in the study. Blood was drawn 1, 2, 4, 8, and 12 h after administration of an oral dose of 1 or 2 g of phenoxymethylpenicillin 1 h after a light breakfast. Antibiotic levels in serum were determined separately by the agar well diffusion method of Bennet et al. (1).

On the basis of the results obtained, doses of 9, 18, and 36 mg of penicillin V per kg of body weight with or without probenecid (25 mg/kg of body weight) were administered by gavage (checked fluoroscopically) to groups of three nonfasted rabbits. Ear vein blood was drawn from each rabbit at 1, 2, 4, 8, and 12 h after treatment for measurement of concentrations of penicillin V in serum.

Assessment of prophylactic activity. Sterile vegetations were produced in New Zealand White rabbits weighing 2.9 to 3.6 kg by the procedure described by Perlmutter and Freedman (10) as modified by Durack and Beeson (4). Briefly, a polyethylene catheter is introduced into the right carotid artery and advanced until it reaches the aortic valve; and the catheter is then secured in place for the duration of the experiment.

* Corresponding author.
Antibiotic susceptibility testing. The MIC and MBC of penicillin V were 0.04 and 0.08 μg/ml, respectively. The in vitro rates of killing of S. sanguis are shown in Fig. 1. Concentrations of 1 and 10 μg of penicillin V per ml killed 5 × 10^5 CFU in 14 h. Thus, this strain was equally susceptible to penicillin V and amoxicillin in vitro (11).

Levels of antibiotics in serum of rabbits and humans. With administration of oral penicillin V (36 or 18 mg/kg of body weight) plus probenecid (25 mg/kg of body weight) to rabbits, the peak levels of antibiotic in serum were 10.4 ± 4.8 and 6.7 ± 3.4 μg/ml, respectively. Both these peak levels and subsequent levels were approximately similar to those achieved in healthy human adults after an oral dose of 2 or 1 g of penicillin V, respectively (Table 1).

Efficacy of prophylactic regimens. The results for the various experimental groups are detailed in Table 2. Although all untreated animals inoculated with 10^5 or 10^6 CFU exhibited IE at necropsy, only 53% (8 of 15; P < 0.01) of the recipients of 10^5 CFU exhibited IE.

The PCN-36/18 regimen provided full protection against challenge with all inocula used. All rabbits treated with the other prophylactic regimens before challenge with 10^6 CFU had sterile vegetations at necropsy, but protection diminished with increasing inoculum size. Among the three regimens which achieved only partial protection, PCN-36 and PCN-18/9 were equally effective and did better than the PCN-18 regimen when rabbits were challenged with 10^6 CFU (P < 0.05 for both regimens). The mean bacterial densities in vegetations of the penicillin-treated rabbits in which IE developed were significantly lower than those in control rabbits challenged with the same inoculum (P < 0.05 for each inoculum).

All untreated animals with culture-positive vegetations also had positive blood cultures. In contrast, S. sanguis was recovered from the blood of only 42 of 54 rabbits in which attempted prophylaxis with the PCN-36, PCN-18, or PCN-18/9 regimen had failed.

DISCUSSION

In 1984, the AHA published its revised recommendations for prophylaxis against IE, suggesting penicillin V orally in a 2-g dose 1 h before dental procedures plus a 1-g dose 6 h afterwards (13). This recommendation was inspired by early experimental streptococcal endocarditis studies in the rabbit which showed that penicillin had preventive effectiveness provided that an early high level in serum followed by at least 9 h of bactericidal action were achieved (5). However, recent data suggest that single intravenous doses of vancomycin (2), amoxicillin (7), penicillin (6), and even bacteriostatic agents (8) protect a considerable number of animals against viridans group streptococcal endocarditis in the absence of bacterial killing, provided that the infective inoculum is lower than the minimum able to induce IE in 90% of control animals. We have found (11) that a single high-concentration oral dose of amoxicillin, probably acting by an alternative prophylactic mechanism(s), achieves full protection against low-concentration inocula of a highly susceptible strain of S. sanguis simulating bacteremia induced by dental work. However, when the maintenance of

Statistical evaluation. The χ^2 test with the Yates’ correction and Student’s unpaired t test were used for statistical comparisons with control groups and between the different prophylactically treated groups.

FIG. 1. Bactericidal activity of penicillin V (PCN) against S. sanguis. Each colony count represents the mean of three determinations. The units on the vertical axis are log_{10} CFU per milliliter.

At 72 h after catheterization, groups of 6 to 10 rabbits were inoculated via the marginal ear vein with approximately 10^6, 10^7, or 10^8 CFU of S. sanguis. In each of 30 sessions, one or two rabbits were randomly assigned to a control group, and the remainder were assigned to a prophylactic regimen. Inocula were prepared from an 18-h culture as described previously (11).

One hour before challenge, subgroups of 15 rabbits each received one of the following regimens (probenecid was always used at 25 mg/kg of body weight): (i) a single oral dose of penicillin V (36 mg/kg of body weight) plus probenecid (regimen PCN-36); (ii) regimen PCN-36 followed by an oral dose of penicillin V (18 mg/kg of body weight) plus probenecid, with a 7-h interval between doses (regimen PCN-36/18); (iii) a single oral dose of penicillin V (18 mg/kg of body weight) plus probenecid, with a 7-h interval between doses (regimen PCN-18/9). Regimen PCN-9 was penicillin V (9 mg/kg) plus probenecid.

Rabbits were sacrificed by 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- 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 for enumeration of colonies. Vegetations were considered sterile in the absence of growth on the plate containing the undiluted homogenate.

Downloaded from http://aac.asm.org on October 27, 2017 by guest
amoxicillin levels in serum was lengthened by administration of a second oral dose, protection against high-grade bacteremias was significantly enhanced. The success of these experiments called for comparative experimental studies on the efficacy of one or two orally administered penicillin V doses comparable to that currently recommended by the AHA.

It is unlikely that successful prophylaxis against *Streptococcus sanguis* IE with a single oral dose of penicillin V (PCN-36 and PCN-18 regimens) was achieved through bacterial killing, because it took 14 h for bactericidal concentrations to kill this strain in vitro. Reduction of protection with increasing inoculum concentrations, which similarly occurred when a single high-concentration oral dose of amoxicillin was used (11), also suggests that prevention of IE was achieved by a mechanism(s) other than bacterial killing (7).

When rabbits were challenged with $10^7$ or $10^8$ CFU of *S. sanguis*, the PCN-36/18 regimen (which induced bactericidal levels in serum for about 14 h) was significantly more effective than the other regimens, protecting all animals again even the highest-concentration inoculum. This successful prophylaxis might be related to the achievement of bacterial killing only when the PCN-36/18 regimen was administered, being remarkable its full protection in this highly susceptible endocarditis model since the serum levels achieved were roughly similar to those expected in humans. Moreover, this regimen seems safe, since halving the dose or administering only a single high-concentration dose of penicillin V protected rabbits acceptably well against the lower inocula, which simulated bacteremia after dental work.

As occurred with oral prophylaxis with amoxicillin (11), mean bacterial densities in vegetations of treated rabbits in which IE developed were lower than in control rabbits challenged with the same inoculum, suggesting a possible postantibiotic effect in vivo (3).

Recent studies (6) have shown that intravenous sodium penicillin (60 mg/kg of body weight), which produced measurable concentrations in serum for about 2 h, reduced the incidence of IE from 95 to 20% when the 90% infective dose was used. Our work substantiates the fact that concentrations of drug insufficient to produce bacterial killing in vitro may still produce a beneficial prophylactic effect in vivo: such an effect was seen with both prophylactic regimens of single-dose oral phenoxymethylpenicillin which we tested. It also shows that lengthening the maintenance of bactericidal levels of penicillin enhances protection against penicillin-susceptible strains, ensuring the clinical safety of the double-high-concentration oral regimen currently recommended by the AHA. Although oral administration of amoxicillin achieves higher and more sustained levels of antibiotic in serum than phenoxymethylpenicillin (12), we have found no differences between these drugs in this experimental model of endocarditis when administered at dosages recommended by the Working Party of the British Society for Antimicrobial Chemotherapy (15) and the AHA (13). Further work evaluating protection against less susceptible strains is needed to definitively elucidate the relative merits of each antibiotic.

ACKNOWLEDGMENTS

We thank J. Liñares for providing the strain of *S. sanguis* used throughout the study and G. Prats for helpful discussions.

This work was supported by grant 85/1299 from the Fondo de Investigaciones de la Seguridad Social, Spain.

LITERATURE CITED


---

TABLE 1. Penicillin V levels in serum after oral administration to nonfasting rabbits and human volunteers

<table>
<thead>
<tr>
<th>Time after dose (h)</th>
<th>PCN-9</th>
<th>PCN-18</th>
<th>PCN-36</th>
<th>Humans 1 g</th>
<th>Humans 2 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6 ± 2.1</td>
<td>6.7 ± 3.4</td>
<td>10.4 ± 4.8</td>
<td>6.2 ± 2.3</td>
<td>12.4 ± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>2.4 ± 1.6</td>
<td>4.0 ± 2.1</td>
<td>7.0 ± 2.6</td>
<td>3.4 ± 2.3</td>
<td>7.0 ± 2.2</td>
</tr>
<tr>
<td>4</td>
<td>0.8 ± 0.5</td>
<td>1.2 ± 1.1</td>
<td>2.5 ± 1.2</td>
<td>1.2 ± 0.9</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0.5 ± 0.1</td>
<td>0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation of the levels in serum determined for at least three rabbits or three healthy human volunteers. Regimens are explained in the text. For humans, penicillin V was used at 1 or 2 g as indicated.

---

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

<table>
<thead>
<tr>
<th>Regimen*</th>
<th>Inoculum (CFU)</th>
<th>No. of sterile vegetations (n = 15)</th>
<th>Mean log$_{10}$ CFU/g of culture per positive vegetation ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no antibiotic)</td>
<td>$10^4$</td>
<td>7</td>
<td>7.64 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>0</td>
<td>7.82 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>0</td>
<td>7.87 ± 0.52</td>
</tr>
<tr>
<td>PCN-36</td>
<td>$10^4$</td>
<td>15</td>
<td>3.66 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>10</td>
<td>3.93 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>5</td>
<td>3.93 ± 0.40</td>
</tr>
<tr>
<td>PCN-36/18</td>
<td>$10^4$</td>
<td>15</td>
<td>4.21 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>15</td>
<td>3.98 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>15</td>
<td>4.06 ± 0.55</td>
</tr>
<tr>
<td>PCN-18</td>
<td>$10^4$</td>
<td>15</td>
<td>4.13 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>$10^6$</td>
<td>10</td>
<td>4.13 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>6</td>
<td>4.13 ± 0.49</td>
</tr>
</tbody>
</table>

* See Materials and Methods for details of regimens.
ENDOCARDITIS PROPHYLAXIS WITH PENICILLIN V


