The in vivo relevance of the paradoxical bactericidal effect (the Eagle effect) is not evident. We found in vitro a paradoxical bactericidal effect of amoxicillin on 2 strains of nontoxigenic Corynebacterium diphtheriae. Then, using an experimental rabbit model of endocarditis, we evaluated the in vivo relevance of this phenomenon. Rabbits were assigned to the following groups: no treatment (control group), continuous amoxicillin infusion simulating a dosage of 200 mg/kg/day in humans, and continuous amoxicillin infusion simulating a dosage of 20 mg/kg/day in humans. The low dosage (20 mg/kg/day) was significantly more effective than the high dosage (200 mg/kg/day) against both strains (P < .025), confirming the paradoxical bactericidal effect observed in vitro.

Nontoxigenic Corynebacterium diphtheriae (NTCD) is considered to be a reemerging pathogen. The incidence of NTCD endocarditis has increased in France: 4 cases were reported before 1986 [1–3], and then 15 cases were reported between 1987 and 1993 [4]. Similarly, of the 60 cases of NTCD endocarditis reported in the world, 50% have been reported since 1997. The mortality rate is relatively high (27%) despite the absence of severe immunosuppression and the availability of a “well-adapted” antibiotic treatment (usually a β-lactam associated with an aminoglycoside). All the French strains were susceptible to amoxicillin, but a paradoxical bactericidal effect (PBE)—described by Eagle and Musselman in 1948 [5] as bactericidal activity that decreases when the concentration of the antibiotic increases—on 2 strains was observed when therapeutic concentrations of amoxicillin were used. In vitro, a PBE on many bacteria has been observed. Therefore, hypothetically, the high mortality rate associated with NTCD endocarditis could be explained by a poor effective bactericidal activity because of a PBE of amoxicillin. The aim of our study was to evaluate the in vivo relevance of the PBE in an experimental rabbit model of NTCD endocarditis.

MATERIALS AND METHODS

Strains 452-92 and 657-93 of NTCD biotype mitis were obtained from the Pasteur Institute. These 2 strains were originally isolated from bacteremic patients, one of whom had endocarditis caused by strain 657-93. The in vitro bactericidal activity of amoxicillin was determined by macrodilution testing in Mueller-Hinton broth (MHB).

C. diphtheriae inocula were prepared by using a Columbia blood agar plate culture incubated overnight at 37°C. Several bacterial colonies were added to MHB that contained amoxicillin. The final bacterial inoculum was 1 × 10^7 cfu/mL, and the final concentration of amoxicillin was 0.06–512 mg/L. After 24 h of incubation at 37°C, the MIC was defined as the lowest concentration of amoxicillin preventing turbidity. Viable bacteria in each glass tube were then counted by subculturing 0.050-mL portions of 10-fold dilutions from each tube. The spiral inoculator system (Spiral System Instruments; Interscience) was used to distribute the septic solution on the agar surface and allowed the detection of antibiotic carryover. In this system, a variable cam-activated syringe dispenses the culture from the center to the edge of the plate in a logarithmically decreasing quantity in the form of an Archimedes spiral. The culture plates were incubated for 48 h at 37°C before viable bacteria were counted. Antibiotic carry-
over could be detected by the absence of colonies in the center of the culture plates.

The in vivo bactericidal activity of amoxicillin was studied using an experimental rabbit model of endocarditis that has been described elsewhere [6, 7]. New Zealand White female rabbits weighing ~2.5 kg were used. The rabbits were placed under general anesthesia, and a polyethylene catheter was positioned through the aortic valve to provoke valvular lesions. The catheter was left in place throughout the study. The next day, each rabbit was inoculated intravenously with 10⁶ cfu of an NTCD strain, to elicit NTCD endocarditis. One day later, rabbits were treated for a 24-h period by intravenous amoxicillin, with simulation of human pharmacokinetics. Each rabbit was randomly assigned to 1 of the following groups: no treatment (control group), continuous amoxicillin infusion simulating a dosage of 20 mg/kg/day in humans (CI20 group), and continuous amoxicillin infusion simulating a dosage of 20 mg/kg/day in humans (CI200 group). The mean serum concentrations of amoxicillin obtained from rabbits were 31 mg/L in the CI200 group and 3 mg/L in the CI20 group. Continuous 24-h venous infusion was administered by use of electric syringe pumps and catheters inserted into a marginal ear vein. Rabbits were euthanized by use of an intravenous bolus of 100 mg of thiopental either before treatment (control group) or 24 h after the beginning of treatment. Previously, we had euthanized 7 untreated rabbits (4 infected with strain 452-92 and 3 infected with strain 657-93) >48 h after inoculation, to verify that the infection was not cleared spontaneously. Thus, a relevant evaluation of the antibiotic regimens in which the number of surviving bacteria before and after treatment could be compared was possible. Aortic valve vegetations were excised, weighed, and homogenized in 0.5-mL physiologic serum. Viable bacteria were counted by subculturing 0.05-mL portions of pure and 100-fold dilutions of the vegetation solutions. Before the viable bacteria were counted, the culture plates (Columbia blood agar) were incubated for 48 h at 37°C. The number of viable bacteria was expressed as log₁₀ colony-forming units/g of vegetation. Carryover was ruled out, as described above. The lower limit of detection for bacterial titers in vegetations was 10 cfu/vegetation (corresponding to 1.46 log₁₀ cfu/g of vegetation in a 35-mg vegetation). The value assigned to the culture-negative vegetations was the lower limit of detection.

RESULTS

The MICs were 0.125 mg/L for strain 452-92 and 0.06 mg/L for strain 657-93. The bactericidal activity of amoxicillin increased when the concentration increased from the MIC to ~2 mg/L and then decreased when the concentration increased to >8 mg/L (figure 1). Thus, amoxicillin had a PBE on both strains of NTCD in vitro.

The results of amoxicillin treatments of NTCD endocarditis are shown in table 1 and figure 2. For each NTCD strain, the dosage of 20 mg/kg/day was significantly more efficient than was the dosage of 200 mg/kg/day (P = .025 for strain 452-92 and P = .005 for strain 657-93, Wilcoxon rank sum test).

DISCUSSION

We observed in vivo relevance of the in vitro PBE of amoxicillin on 2 strains of NTCD. The bactericidal activity of amoxicillin was higher at the low dosage (CI20) than at the high dosage (CI200). The dosage of 200 mg/kg/day is usually prescribed in human endocarditis, and some intensive care physicians use such high doses to treat other severe infections. The dosage of 20 mg/kg/day is not prescribed in clinical practice. Because our model simulates an acute endocarditis, the amoxicillin dosages are probably not applicable for use in subacute endocarditis, because amoxicillin diffusion in vegetations might be weaker. Nevertheless, the results of our study suggest that, in some pa-

![Image](https://academic.oup.com/jid/article-lookup/10.1093/jid/191/12/2118)

Table 1. Bacterial titers in vegetations after use of different dosages of amoxicillin in an experimental rabbit model of non-toxigenic Corynebacterium diphtheriae endocarditis.

<table>
<thead>
<tr>
<th>Bacterial titer</th>
<th>C. diphtheriae (strain 452-92)</th>
<th>C. diphtheriae (strain 657-93)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.50 ± 0.40 (7)</td>
<td>8.37 ± 0.60 (11)</td>
</tr>
<tr>
<td>20 mg/kg/day amoxicillin</td>
<td>2.61 ± 0.27 (13)*</td>
<td>3.01 ± 0.86 (18)b</td>
</tr>
<tr>
<td>200 mg/kg/day amoxicillin</td>
<td>4.28 ± 1.72 (6)</td>
<td>5.11 ± 0.91 (10)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD log₁₀ cfu/g of vegetation (no. of rabbits).

* Significant difference vs. the 200 mg/kg/day amoxicillin group (P< .025, Wilcoxon rank sum test).

b Significant difference vs. the 200 mg/kg/day amoxicillin group (P< .005, Wilcoxon rank sum test).
Figure 2. Bactericidal activity of 2 amoxicillin dosages in an experimental rabbit model of nontoxigenic Corynebacterium diphtheriae endocarditis. The bactericidal activity is significantly greater with the 20 mg/kg/day vs. the 200 mg/kg/day dosage for strain 452-92 (P < .025, Wilcoxon rank sum test) and strain 657-93 (P < .005, Wilcoxon rank sum test).

Two experimental studies have demonstrated an in vivo relevance of the PBE: Proteus vulgaris with cefmenoxime in a mouse model of peritoneal infection [8] and Staphylococcus aureus with cloxacillin in a rat model of endocarditis [9]. In humans, the PBE has not been studied. Nevertheless, an observation suggests that the PBE may be clinically relevant [10]. A man with an α-hemolytic Streptococcus endocarditis was treated with benzylpenicillin alone. On the 12th day of treatment, the patient was still ill, and the serum bactericidal activity was weak. When the dosage was lowered from 12 million U/day to 6 million U/day, the patient’s clinical status and serum bactericidal activity improved. Unfortunately, the clinical status of the patient (fever, etc.) was not precisely defined in the study.

The mechanism of the PBE has not been clearly established. High concentrations of penicillin are known to decrease protein and RNA synthesis in a dose-dependent manner in streptococci [11]. Defective autolytic activity has been implicated in a study of Enterococcus faecalis [12].

In conclusion, the possibility of a PBE must be considered in the treatment of NTCD endocarditis and may explain the possible failure of high-dose penicillin treatment. Physicians should respect the recommended maximum doses of first-intention antibiotics (instead of increasing the dose in the treatment of a severe infection), because the PBE, which is relevant in some animal models, could be relevant in humans.

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References