Different Aminoglycoside-Resistant Phenotypes in a Rabbit
*Staphylococcus aureus* Endocarditis Infection Model

N. Asseray,1 J. Caillon,1 N. Roux,1 C. Jacqueline,1 R. Bismuth,2 M. F. Kergueris,3 G. Potel,1,* and D. Bugnon1

Laboratoire d’Antibiologie and Laboratoire de Toxicologie,3 UER de Médecine, Nantes, and
Service de Bactériologie, Hospital Pitié Salpêtrière, Paris,3 France

Received 26 June 2001/Returned for modification 18 November 2001/Accepted 25 January 2002

The impact of different types of enzymatic resistance on the in vivo antibacterial activity of aminoglycosides (amikacin, gentamicin, and netilmicin) was studied in the rabbit endocarditis model with four strains of *Staphylococcus aureus*. Animals were treated in a manner simulating the administration of a single daily human dose. Amikacin had no effect on the three kanamycin-resistant strains despite apparent susceptibility in the disk diffusion test. Gentamicin appears to be the preferable aminoglycoside for treatment of staphylococcal infections.

More than 40% of the *Staphylococcus aureus* strains isolated in hospitals are resistant to methicillin and show associated resistance to other antibiotics, especially aminoglycosides (8, 9, 11). The treatment of methicillin-resistant *S. aureus* infections is based principally on glycopeptides (mainly vancomycin). When combination therapy is required, aminoglycosides are used because of their rapid and intense killing power (16, 17, 21). Enzymatic inactivation is the major mechanism of resistance to aminoglycosides (15, 19). Three types of enzymes, APH(3’), ANT(4’), and APH(2’)-AAC(6’) are involved, respectively, in resistance to kanamycin (K’), kanamycin and tobramycin (KT’), and kanamycin, tobramycin, and gentamicin (KTG’). Other aminoglycosides are also modified by these enzymes; namely, netilmicin is modified by APH(2’)-AAC(6’) and amikacin is modified by all three enzymes, whereas their bacteriostatic activity against *S. aureus* is preserved (1, 2; R. Bismuth, J. R. Pirault, H. Drugeon, and P. Courvalin, 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 705, 1989).

The purpose of this in vivo study in a rabbit *S. aureus* endocarditis infection model was to assess the impact of these enzymes on the antibacterial activity of amikacin and gentamicin, alone or in combination with a cell wall-active antibiotic (such as vancomycin), and to determine whether a disk diffusion test can indicate the most suitable aminoglycoside with which to combat a staphylococcal infection.

Four *S. aureus* strains were studied; they were isolated from different clinical samples kindly supplied by R. Bismuth. Two of them were methicillin resistant (MecA’), and the other two were methicillin susceptible (MecA’). A previous hybridization study (R. Bismuth, unpublished data) showed that the plasmid content of these strains coded for enzymes modifying aminoglycosides. One carried no enzyme and displayed a susceptible wild-type phenotype (S-SA, methicillin resistant). The other three produced an enzyme: APH(3’)-producing K-SA (methicillin susceptible), ANT(4’)-producing KT-SA (methicillin resistant), and AAC(6’)-APH(2’)-producing KTG-SA (methicillin susceptible). Disk diffusion tests were performed in accordance with the standard agar plate method recommended by a committee of the French Society for Microbiology (5). Mueller-Hinton media, in which calcium (50 mg/liter) and magnesium (25 mg/liter) had been adjusted, were seeded with a 10⁶-CFU/ml suspension and read after a 24-h incubation at 37°C. The MIC was determined by microdilution in liquid medium (6) after a 24-h incubation at 37°C. Strains were rated as susceptible, intermediate, or resistant with respect to zone diameters and critical concentrations (5). The same method was used to determine the MIC of vancomycin for each strain.

Aortic endocarditis (14) was induced in female New Zealand rabbits by insertion of a polyethylene catheter into the left ventricle, followed 24 h later by intravenous inoculation of 10⁸ CFU of each *S. aureus* strain. Twenty-four hours after inoculation, control animals were sacrificed and treatments for a 48-h period were begun. Five treatments were studied: gentamicin (Shering-Plough Laboratories, Levallois-Perret, France), amikacin (Bristol-Myers Squibb Laboratories, Paris-La Défense, France), vancomycin (Lilly Laboratories, St-Cloud, France), and the combinations gentamicin-vancymycin and amikacin-vancymycin. Netilmicin (Shering-Plough Laboratories, Paris, France) was only modified by the bifunctional enzyme and was thus tested only on the KTG-SA strain in order to validate the results observed with gentamicin on this strain. Aminoglycosides were administered to rabbits in a single daily dose, in agreement with current recommendations concerning the best dosage regimen for these drugs (7, 10, 12, 18), simulating the kinetics of a human dosage of amikacin of 15 mg/kg/day, of gentamicin of 3 mg/kg/day, or of netilmicin of 6 mg/kg/day, by using a computerized system of automatic syringes managed by software to adapt the infusion rate. Vancomycin was administered at a constant intravenous infusion rate in order to simulate a total human dose of about 30 mg/kg, allowing the achievement of a steady-state concentration of around 25 mg/liter of serum. After sacrifice, thoracotomy, and cardiecotomy,
endocarditis vegetations were removed, pulverized, stored in ice, and ultimately ground in a 0.5-ml volume of a saline solution. The homogenate obtained was then spread on Trypcase-soja agar plates in pure form or after a 1:100 or 1:1,000 dilution. The homogenate obtained was then spread on Trypcase-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diam (mm), MIC (mg/liter), susceptibility*</th>
<th>Vancomycin MIC (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amikacin</td>
<td>Tobramycin</td>
</tr>
<tr>
<td>S-SA</td>
<td>26, 1, S</td>
<td>26, 0.5, S</td>
</tr>
<tr>
<td>K-SA</td>
<td>24, 1, S</td>
<td>25, 0.25, S</td>
</tr>
<tr>
<td>KT-SA</td>
<td>23, 8, S</td>
<td>6, 16, R</td>
</tr>
<tr>
<td>KTG-SA</td>
<td>21, 8, S</td>
<td>6, 64, R</td>
</tr>
</tbody>
</table>

* S, susceptible; R, resistant.

synergy was exhibited when vancomycin was combined with gentamicin or amikacin. Vancomycin alone was active in vivo only against the S-SA and K-SA strains.

The results of antibiotic assays for the peak and trough concentrations of aminoglycosides and the steady-state concentration of vancomycin confirm that the objectives set were reached. The peak and trough concentrations in serum, respectively, were as follows: amikacin, 49.5 ± 1.6 and 1.2 ± 0.5 mg/liter; gentamicin, 15.8 ± 1.2 and 1.2 ± 0.2 mg/liter; netilmicin, 45.7 ± 4.3 and 0.3 ± 0.1 mg/liter. The steady state concentration of vancomycin in serum was 22.3 ± 8.7 mg/liter.

Under our experimental conditions, the apparent aminoglyco-

- **TABLE 2.** Results obtained in vivo after 48-h treatment of the four strains studied

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Mean log CFU/g of vegetation ± SD (no. of rabbits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-SA</td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 0.5 (7)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.4 ± 0.4 (5)*</td>
</tr>
<tr>
<td>Amikacin</td>
<td>5.5 ± 0.4 (5)*</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>ND</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>3.3 ± 1.5 (5)*</td>
</tr>
<tr>
<td>Vancomycin + Gentamicin</td>
<td>3.0 ± 0.9 (7)*</td>
</tr>
<tr>
<td>Vancomycin + Amikacin</td>
<td>2.6 ± 0.8 (4)*</td>
</tr>
</tbody>
</table>

* P < 0.0001 versus control and amikacin (Sheffe’s test after ANOVA).
* P < 0.0001 versus control and gentamicin.
* P = 0.0009 versus control and P = 0.0004 versus amikacin.
* P = 0.03 versus control.
* No difference between vancomycin alone and each combination.
* No difference between gentamicin alone and each combination.
* No difference versus control.
* ND, not done.
* NI, not interpretable (all of the animals in this group died).
therapeutic effect during clinical applications, even though studies indicate that bacteria are just as sensitive to the antibiot-
cic, which confirm that the loss of bactericidal activity in vitro
corresponds to that in vivo, tend to validate this line of rea-
soning. The loss of kanamycin activity observed in disk dif-
fusion tests was predictive of a loss of amikacin efficacy in vivo,
despite the apparent susceptibility of bacteria to this amin-
glycoside. The absence of gentamicin activity in disk diffusion
tests was predictive of resistance to gentamicin, amikacin, and
netilmicin.

In the present study, the human pharmacokinetics of ami-
oglycosides was simulated in the rabbit (18). Thus, the results
obtained are more predictive of the efficacy of treatments used
in severe septicemic S. aureus infection. Based on the results
of this study, a clinical indication of amikacin in the context of an
acute infection with S. aureus cannot be maintained, despite
the apparent sensitivity shown in disk diffusion tests. Con-
versely, gentamicin can provide a real therapeutic benefit
against strains with a K' or KT' phenotype. Moreover, the
greater in vivo efficacy of gentamicin against the strain with a
sensitive phenotype seems to confirm that this aminoglycoside
is preferable for the treatment of staphylococcal infections. To
our knowledge, this is the first in vivo evidence of the validity
of the interpretation proposed by Bismuth et al. (1; Bismuth et al.,
29th ICAAC). However, the resistance of strains with a
KTG' phenotype to gentamicin precludes the use of an ami-
glycoside against infections with these strains.

REFERENCES
MPC-Vigot, Paris, France.
Maloine, Paris, France.
concentration on reaction rates of aminoglycoside-modifying enzymes. An-
4. Bugnon, D., G. Potel, J. Caillon, D. Baron, H. B. Drugeon, M. F. Kergueris,
and P. Feigel. 1998. In vivo simulation of human pharmacokinetics in the
niques. Préparation d’une gamme d’antibiotiques. Détermination de la con-
centration minimale inhibitrice en milieu liquide, p. 189–192. In P. Courvalin
et al. (ed.), L’antibiogramme. MPC-Vigot, Paris, France.
namic requirements for the control of experimental endocarditis. An-
8. Lelièvre, H., G. Lina, M. E. Jones, C. Olive, F. Forey, M. Rousset-Delval-
le, M. H. Nicolas-Chanoine, et al. 1999. Emergence and spread in French
hospitals of methicillin-resistant Staphylococcus aureus with increasing sus-
3457.
Jarlier. 1998. Characterization of gentamicin-susceptible strains of methi-
therapy: Importance of the ratio of peak concentration to minimal inhibitory
consensus review of microbiology, pathogenesis, and epidemiology, with
on the safety and efficacy of aminoglycosides given either once daily or as
necity for aminoglycoside-modifying enzymes in gram-positive cocci. Antimi-
14. Perlman, B., and I. Freedman. 1971. Experimental endocarditis II. Staph-
hylococcal infection of the aortic valve following placement of a polyethylene
aminosides, p. 23–34. In P. Courvalin, H. B. Drugeon, J. P. Flandrois, and F.
daily aminoglycoside in the treatment of Enterococcus faecalis endocarditis:
characterization of aminoglycoside-modifying enzymes from Staphylococcus
aureus and Staphylococcus epidermidis. Antimicrob. Agents Chemother. 25:
754–759.
genes encoding aminoglycoside-modifying enzymes. Antimicrob. Agents
Chemother. 42:483.
1995. Antiobiotic treatment of adults with infective endocarditis due to strep-
tococci, enterococci, staphylococci, and HACEK microorganisms. JAMA
274:1706–1713.