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The aim of the present study was to characterize the pharmacokinetic-pharmacodynamic relationship of GV143253A, a novel trinem anti-methicillin-resistant Staphylococcus aureus (MRSA) agent active against gram-positive cocci, including multidrug-resistant clinical isolates. An in vitro pharmacodynamic study with methicillin-susceptible S. aureus (MSSA) and MRSA has shown that the duration of exposure to GV143253A rather than its concentration is the major determinant of the extent of bacterial killing. In vivo findings were confirmed by use of a neutropenic murine model of thigh infection caused by MSSA ATCC 25923. From the dose-response curves, the static doses extrapolated for three different dosing intervals showed that more frequent dosing of GV143253A was more effective than less frequent dosing. A pharmacokinetic-pharmacodynamic analysis demonstrated that only the time during which the drug concentration exceeded the MIC (t>MIC) correlated with in vivo GV143253A activity. The value of t>MIC required to achieve a bacteriostatic effect in a thigh infection of neutropenic animals was 20% (95% confidence interval [CI], 18 to 22%) of the dosing interval. This result is similar to those reported in the literature for carbapenems and for GV104326A, another novel trinem compound. In addition, in order to compare the therapeutic efficacy of GV143253A to that of vancomycin in a thigh infection caused by MRSA in immunocompetent mice, suitable dosing regimens were designed on the basis of previous pharmacokinetic-pharmacodynamic findings for GV143253A and on the human pharmacokinetic profile of the glycopeptid. Although the pharmacokinetic profiles of the two agents were completely different, GV143253A showed good efficacy comparable to that of vancomycin, reducing by 4 log units the bacterial counts in the thighs of treated mice relative to untreated infected animals after 48 h of therapy. The results suggest that if the time of exposure to the pathogen above the MIC is at least 30% of the dosing interval, GV143253A could have a role in the clinical treatment of infections caused by MRSA, which is difficult to eradicate with current antibiotics.

Staphylococcus aureus is one of the most common pathogens of community-acquired and nosocomially acquired infections and represents a current serious problem (14). Moreover, S. aureus bacteria showing a phenotype of methicillin resistance and, more recently, of multidrug resistance have been isolated with increasing incidence in hospitals. The antistaphylococcal β-lactam antibiotics are the most active agents available for the treatment of methicillin-susceptible S. aureus (MSSA) infections, while vancomycin is considered the drug of choice to cure infections caused by methicillin-resistant S. aureus (MRSA). However, the use of this glycopeptid presents some problems due to its static mode of action, which could affect the duration of therapy, to significant adverse effects, and to failure to cure infections caused by vancomycin-intermediate strains reported in many different regions (8, 9).

GV143253A is a novel trinem with a very interesting spectrum of activity against gram-positive cocci, including resistant clinical isolates. The activity against MRSA is due to the high level of affinity of GV143253A for PBP2* (2). In previous in vitro and in vivo studies, GV143253A compared favorably with vancomycin (16). In particular, it showed a high level of efficacy in the cure of systemic and localized infections caused by MRSA, such as soft tissue infections, and severe diseases, such as endocarditis, in rats.

Since the integration of the pharmacokinetic and pharmacodynamic properties of novel compounds makes it possible to identify the surrogate marker responsible for efficacy and to predict the efficacious dose in patients, in vitro and in vivo pharmacokinetic-pharmacodynamic characterizations of GV143253A have been carried out.

It is known from the literature that the time above the MIC (t>MIC) required for efficacy can vary among the different classes of β-lactams. In general, for cephalosphorins, penicillins, and carbapenems, the t>MIC of the free drug in serum needs to be maintained for about 40 to 50%, 25 to 35%, and 18 to 27% of the dosing interval, respectively, in order to provide a static effect in neutropenic animal models of infection, regardless of the type of the etiological pathogen (3, 5, 10; W. A. Craig, S. Ebert, and Y. Watanabe, Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 86, 1993). Moreover, Andes et al. (D. Andes, O. Vesga, and W. A. Craig, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 36, 1996) demonstrated that the in vivo pharmacodynamic activity of GV104326A, a novel trinem compound, is similar to that of carbapenems.

The objectives of the present study were to investigate the in vitro pharmacodynamic properties of GV143253A in terms of time-kill curves and to determine the effects of various dosing...
regimens on the in vivo efficacy of GV143253A in order to identify the pharmacokinetic parameter that correlates best with efficacy. In addition, taking into account the pharmacokinetic-pharmacodynamic findings, in order to predict the clinical efficacy of GV143253A relative to that of vancomycin against infections caused by MRSA, a model of thigh infection in immunocompetent mice with selected dosing regimens for the two antibiotics was used.

**MATERIALS AND METHODS**

**Antibiotics.** GV143253A was synthesized at GlaxoWellcome SpA (Verona, Italy). Vancomycin (Sigma Chemical Co.) was used for comparative in vitro studies. Antibiotic solutions were prepared in sterile 0.1 M potassium phosphate buffer (pH 7.4) or according to the manufacturers’ recommendations on the day of use.

**Bacteria.** For in vitro and in vivo pharmacodynamic studies, the following strains were used: *S. aureus* ATCC 25923 (methicillin susceptible) and *S. aureus* 4538 and 4543 (methicillin-resistant clinical isolates from Japan). The strains were maintained as lyophilized cultures and, before use, were subcultured twice on blood agar base (BBL, Cockeysville, Md.).

**Susceptibility studies.** MICs were determined by the agar dilution technique with Mueller-Hinton agar (MHA; Difco) according to the technical procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS M7-A4) (15). The medium was supplemented with 2% NaCl for recommendations by the National Committee for Clinical Laboratory Standards for MRSA. The inoculum was prepared by dilution of overnight cultures to obtain the inoculum size was approximately 10^6 CFU/ml. Subcultures were twice on blood agar base (BBL, Cockeysville, Md.).

**Pharmacokinetics.** For in vitro and in vivo pharmacodynamic studies, the following antibiotics were used: aminoglycosides (gentamicin, amikacin, tobramycin, and netilmicin), vancomycin, teicoplanin, minocycline, ticarcillin, and imipenem. The two antibiotics was used.

**Killing curves.** Bactericidal activity was assessed by measuring changes in the viable counts of bacteria exposed to various concentrations of GV143253A and vancomycin over a 6-h period.

**Plasma drug concentration-time data.** Concentration-time curves were described by a one-compartment model with first-order input and output. For in vitro and in vivo pharmacodynamic studies, the following equations were used:

**Thigh infection.** For thigh infection, fluorouracil-anesthetized neutropenic mice were inoculated intramuscularly in both hind legs with 0.125 ml of an exponential-phase culture (optical density at 590 nm, 0.3) of *S. aureus* ATCC 25923 grown in MHB and washed and resuspended in the same fresh broth to 5.2 × 10^9 CFU/ml. Four mice per group were used. Mice were treated for 24 h with total antibiotic doses ranging from 10 to 1,200 mg/kg. Dosing regimens were selected by dividing 24-h total doses into individual doses to be administered at 3-t (q3h), 6-h (q6h), and 12-h (q12h) intervals. Antibiotics were administered subcutaneously 2-ml volumes, beginning 2 h after thigh infection.

**Bacterial counts.** Bacterial counts were determined at the start of therapy and 24 h after therapy. Mice were sacrificed by cervical dislocation, and the entire thigh region was cleaned, skinned, removed aseptically, and then homogenized in ice-cold 0.9% NaCl with a tissue homogenizer (Ultra Turrax; Janke and Kunkel). Bacterial cell counts were determined on MHA by plating duplicate samples of appropriate dilutions of the homogenate and incubating the plates for 18 to 24 h at 37°C. The detection limit was 1.7 log CFU/g.

**Pharmacokinetic-pharmacodynamic analysis.** The GV143253A dose-response relationship for *S. aureus* was analyzed for each dosing regimen (q3h, q6h, and q12h) by using the following equation:

\[
E = E_{\text{max}} - \left( E_{\text{max}} - E_0 \right) \times \left[ D \left( D + E_{D_{50}} \right) \right]^{-1}
\]

where *E* is the log CFU per gram after 24 h of therapy, *D* is the dose administered for each dosing interval, *E_{\text{max}}* is the log CFU per gram for the control group at the 24 h of therapy, *E_0* is the theoretical log CFU per gram at an infinite dose, and *E_{D_{50}}* is the dose eliciting 50% of the bactericidal activity. For each dosing interval, the static dose of GV143253A (i.e., the dose required to produce a net bacteriostatic effect over 24 h) was calculated.

The GV143253A pharmacokinetic parameters (maximum concentration of drug in serum [C_{\text{max}}, area under the concentration-time curve [AUC], and t1/2-MIC) at all doses considered during the pharmacodynamic analysis were obtained from the simulated profiles. Protein binding of GV143253A has been measured in different species. In particular, the free fractions in humans and mice have been found to be 40 and 28%, respectively. Considering the limited protein binding in both species and the relatively short half-life in mice, pharmacokinetic-pharmacodynamic calculations were made by using the total plasma drug concentration. A preliminary check of the influence of protein binding was done, and the differences found were negligible.

**Efficacy in the thigh infection model.** Immunocompetent mice were inoculated intramuscularly in the hind legs with 0.125 ml of an overnight culture in MHB-2% NaCl of appropriately diluted MRSA 4538 (4.4 × 10^9 CFU/ml) and vancomycin was administered at a dose of 100 mg/kg at 0, 1, 8, 9, 16, and 17 h, while vancomycin was administered at a dose of 300 mg/kg every 12 h.

The efficacy of parenteral therapy was measured in terms of log viable bacteria (CFU per gram of tissue) recovered with respect to recovery in untreated infected animals. The detection limit was 1.7 log CFU/g. The results obtained for each group of animals were evaluated by calculating the geometric mean and standard deviation. Statistical comparisons of viable bacterial counts for the different treated and untreated groups were performed by using Student’s multiple-comparison test. Differences were considered significant when the *P* value was <0.05.
RESULTS

Killing curves. Fig. 1 depicts the time-kill curves derived from testing of GV143253A and vancomycin against MSSA ATCC 25923, MRSA 4538, and MRSA 4543 at the MIC and at four times the MIC.

GV143253A killed efficiently the three isolates even at a low concentration (the MIC), reducing the viable counts of MSSA, MRSA 4538, and MRSA 4543 by 2.7, 2.5, and 3.4 log units after 4 h of incubation, respectively. As expected, GV143253A was more bactericidal than vancomycin at all concentrations tested against all strains. In particular, against MSSA, GV143253A and vancomycin decreased viable bacterial counts by 3.8 and 1.6 log units, respectively, compared to the control, after 6 h of incubation at four times the MIC. Similar findings were observed for MRSA 4538 and MRSA 4543. GV143253A and vancomycin reduced viable bacterial counts for the first strain by 2.8 and 2 log units, respectively, compared to the control, and reduced those for the second strain by 4.3 and 2.8 log units, respectively, compared to the control, after 6 h of incubation at four times the MIC. In all instances, the two antibacterial agents at different concentrations showed similar bactericidal profiles. In fact, saturation of the rate of killing was observed at a concentration near the MIC, and high concentrations did not kill the organisms faster or more extensively than the lowest concentration tested.

These results indicate that, as for other β-lactam antibiotics, the duration of bacterial exposure to GV143253A rather than the drug concentration is the major determinant of the extent of killing.

Pharmacokinetic study. Figure 2 shows the median plasma concentration-time profiles for GV143253A after subcutaneous administration at 10, 200, and 600 mg/kg to neutropenic mice infected in the thighs. The main pharmacokinetic parameters are reported in Table 1. The time to the maximum concentration of the drug in plasma was about 10 min after the drug was administered. The $C_{\text{max}}$ and the AUC increased in proportion with the dose of GV143253A administered, ranging from 12 to 784 μg/ml and from 6.3 to 609 μg·h/ml, respectively. Differences were not statistically relevant.

Pharmacokinetic parameters were previously evaluated with uninfected mice at a single dose of the drug (10 mg/kg). Plasma profiles and derived pharmacokinetic parameters were comparable to those obtained with infected immunocompromised...
mice; therefore, the data generated from infected mice were considered valid for uninfected mice as well.

Correlation of pharmacokinetic parameters with in vivo pharmacodynamic activity. The GV143253A dose-response relationships for each dosing regimen (q3h, q6h, and q12h) are shown in Fig. 3. The estimated static dose (i.e., the dose required to produce a net bacteriostatic effect over 24 h) for each dosing regimen is reported in Table 2.

Visual inspection of the log CFU per gram after 24 h of therapy plotted against \( C_{\text{max}}/\text{MIC} \) and AUC/MIC (Fig. 4A and B) did not suggest any clear correlation. This conclusion was also confirmed by the slopes (−0.0012 and −0.00003 for \( C_{\text{max}}/\text{MIC} \) and AUC/MIC, respectively) of the linear regressions and the low \( R^2 \) values (0.26 and 16.8% for \( C_{\text{max}}/\text{MIC} \) and AUC/MIC, respectively) of the linear regressions.

Visual inspection of the log CFU per gram after 24 h of therapy plotted against \( t_{\text{max}}/\text{MIC} \) (expressed as a fraction of the dosing interval) suggested a good correlation (Fig. 4C). The \( R^2 \) value describing the relationship between \( t_{\text{max}}/\text{MIC} \) and log CFU per gram, obtained by modeling data with the Hill function, was 95%. Figure 4C shows the fitting of data obtained together with the 95% CIs around the function. The values for model parameters are reported in Table 3.

The \( t_{\text{max}}/\text{MIC} \) needed to produce a net static effect was 20%, with a 95% CI of 18 to 22%.

Efficacy in the thigh infection model. The aim of this study was to evaluate, for a mouse soft tissue infection caused by an MRSA strain, the efficacy of GV143253A in comparison with that of vancomycin. Dose regimens were designed by taking into account pharmacokinetic-pharmacodynamic findings for the first drug and the human pharmacokinetic profile obtained with the recommended therapeutic dose for the second drug. For this reason, mice were treated with vancomycin at a dosing regimen able to yield pharmacokinetic-pharmacodynamic parameters equal or even superior to those obtained in humans with the dosing regimen clinically used: 1 g q12h. In order to do this, the values for the pharmacokinetic-pharmacodynamic parameters relative to the dosing regimens for vancomycin in mice and humans were compared (Table 4). A dose of 0.3 g q4h in mice guaranteed superior AUC/MIC and \( C_{\text{max}}/\text{MIC} \) values with respect to those reported in humans and a \( t_{\text{max}}/\text{MIC} \) of 100%.

As pharmacokinetic-pharmacodynamic studies with GV143253A showed that a \( t_{\text{max}}/\text{MIC} \) of 20% was required to achieve a bacteriostatic effect in the thigh infection model with neutropenic animals, a \( t_{\text{max}}/\text{MIC} \) of 30% was considered sufficient to cure an infection caused by MRSA in immunocompetent mice (6, 10). In fact, in this animal model, at least a 1-log-unit drop is considered sufficient to obtain resolution in immunocompromised mice; therefore, in immunocompetent animals, a bacteriocidal effect is guaranteed.

Therefore, on the basis of the pharmacokinetic profile for GV143253A in mice, with the assumption that, as for other \( \beta \)-lactam compounds, the profile does not change significantly from neutropenic to immunocompetent mice, and on the basis of the activity of the drug against MRSA 4538, a suitable dosing regimen was designed: 0.2 g q8h.

The efficacies of the two drugs in terms of reduction of bacterial counts were very similar at 48 h (Fig. 5), with a decrease in bacterial counts of about 4 log units with respect to those in the untreated infected group. GV143253A was clearly as effective as vancomycin for curing soft tissue infections caused by MRSA, despite the fact that the \( t_{\text{max}}/\text{MIC} \) of GV143253A was much lower than that of vancomycin. These results emphasize the fact that the two antibiotics have different in vivo bactericidal properties and pharmacokinetic-pharmacodynamic relationships.

**DISCUSSION**

GV143253A is a novel anti-MRSA trinem that was reported in previous studies to possess in vitro and in vivo potencies comparable to those of vancomycin and is a drug of choice for the cure of infections caused by multidrug-resistant gram-positive pathogens.
In order to select the efficacious dose in patients and to better design clinical trials, pharmacokinetic-pharmacodynamic studies were performed. In addition, pharmacokinetic and pharmacodynamic parameters could help not only to maximize bacteriological cure but also to minimize the potential for the selection and spread of resistance, a particularly relevant problem with pathogens, such as MRSA, that are also multidrug resistant (1, 18).

**TABLE 3.** Correlation of t>MIC with antibacterial activity, analyzed with the Hill function$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SD</th>
<th>% Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{max}$</td>
<td>9.00</td>
<td>0.24</td>
<td>2.7</td>
</tr>
<tr>
<td>EX$_{50}$ (min)</td>
<td>25.3</td>
<td>1.33</td>
<td>5.3</td>
</tr>
<tr>
<td>$E_0$</td>
<td>4.11</td>
<td>0.21</td>
<td>5.2</td>
</tr>
<tr>
<td>Hill coefficient$^b$</td>
<td>3.32</td>
<td>0.51</td>
<td>15.2</td>
</tr>
<tr>
<td>Static t&gt;MIC, % (95% CI)$^c$</td>
<td>20 (18–22)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ The $R^2$ value was 95%.

$^b$ The Hill coefficient accounts for the sigmoid characteristic of the curve.

$^c$ t>MIC needed to produce a net static effect (static log CFU per gram) over 24 h of therapy.

**TABLE 4.** Pharmacokinetic-pharmacodynamic parameters for GV143253A and vancomycin$^a$

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Dosing regimen</th>
<th>AUC/MIC (h)$^b$</th>
<th>$C_{max}$/MIC$^c$</th>
<th>t&gt;MIC (%)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV143253A</td>
<td>Mouse</td>
<td>0.2 g q8h</td>
<td>256</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td>Vancomycin$^e$</td>
<td>Mouse</td>
<td>0.3 g q4h</td>
<td>2,666</td>
<td>417</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>1 g q12h</td>
<td>422</td>
<td>66</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ Determined at the dosing regimen used for thigh infection of mice with MRSA 4538 and at the clinically recommended dosing regimen for vancomycin.

$^b$ The MICs of GV143253A and vancomycin against MRSA 4538 were 2 and 1 µg/ml, respectively.

$^c$ Data were obtained by simulation of the reported dosing regimens on the basis of published pharmacokinetic profiles for vancomycin in mice and humans (7, 11, 12, 17).
Like the other β-lactam antibiotics, GV143253A exhibited minimal in vitro concentration-dependent killing of MSSA and MRSA strains. At concentrations four times the MIC, GV143253A did not kill the organisms more effectively than at lower concentrations (the MIC). The killing activity of GV143253A confirmed its higher bactericidal effect with respect to vancomycin. On the basis of the in vitro findings, the goal of a dosing regimen for this drug is the optimization of the time of exposure.

In order to understand the pharmacokinetic-pharmacodynamic parameters that predict efficacy, dose-response relationships were determined by using a thigh infection model described by Craig et al. and Leggett et al. (4, 13).

The dose-response curves obtained with three different dosing intervals confirmed a higher efficacy of GV143253A at a shorter dosing time interval. In fact, the estimated static dose was reduced about 200-fold at a shorter dosing interval (3 h), suggesting, once again, that the bactericidal effect of GV143253A is time dependent rather than concentration dependent; this finding was consistent with the properties of the β-lactam antibiotics. These findings were then confirmed by a pharmacokinetic-pharmacodynamic analysis of the data. In an analysis of the relationship between each pharmacokinetic parameter (C_{max}/MIC, AUC/MIC, and t>MIC) and the efficacy of GV143253A, only t>MIC showed a good correlation. The value required to achieve a bacteriostatic effect against MSSA in neutropenic mice was 20% (95% CI, 18 to 22%) of the dosing interval, similar to values reported in the literature for carbapenems and for the trinem GV104326A.

The findings of the pharmacokinetic-pharmacodynamic analysis were used to select an optimal dosing regimen to compare the pharmacodynamics of GV143253A with those of vancomycin in a thigh infection model with MRSA and immunocompetent mice. Since a t>MIC of 20% was the value needed to obtain a bacteriostatic effect in the thigh infection model with neutropenic mice, a dosing regimen able to cover a t>MIC of 30% in the same model but with immunocompetent mice was considered sufficient for GV143253A to cure the infection. A different dosing regimen strategy was followed for vancomycin, considering that it is on the market and used at a dose of 1 g q12h to treat the most severe infections. GV143253A and vancomycin showed comparable efficacies in curing infected mice, reducing by 4 log units the bacterial counts in thighs of treated mice with respect to control mice after 2 days of therapy.

In conclusion, because of its wider spectrum of activity with respect to vancomycin, GV143253A has the potential to be used as an additional agent for treating suspected gram-positive (including MRSA) infections, allowing glycopeptides to be reserved for trinem-resistant cases and thereby reducing the increase in glycopeptide resistance development. Moreover, this study emphasizes the importance of a pharmacokinetic-pharmacodynamic analysis for a novel antibiotic not only to select the therapeutic dose for clinical trials in humans but also, in the preclinical phase, to define a suitable dosing regimen to assess the efficacies of novel agents in animals models of infection.

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REFERENCES


