Charge and Lipophilicity Govern the Pharmacokinetics of Glycopeptide Antibiotics

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The pharmacokinetics and urinary excretion of nine glycopeptide antibiotics with diverse pIs (3.8 to 8.5) and lipophilicities were studied. The disposition of the aridicin antibiotics and their hydrolysis products were examined in male CD-1 mice after subcutaneous and intravenous administration and compared with the disposition of teicoplanin, ristocetin, and vancomycin. The total systemic clearance, half-life, volume of distribution, and urinary excretion were highly correlated with pIs. In general, as the pI decreased, the clearance, urinary recovery, and volume of distribution decreased, whereas the half-life increased. With those glycopeptides that had similar pIs, clearance decreased and half-life increased with increasing lipophilicity. The urinary recovery of the glycopeptides decreased with decreasing pI and increasing lipophilicity. Because vancomycin (pI = 8.0) is cleared by glomerular filtration, increased binding to serum is the likely mechanism of reduced renal clearance for glycopeptides with low pIs. These results are consistent with previous findings concerning the correlation of physical-chemical properties and the drug disposition of small organic molecules. Results of these studies also indicate that desirable pharmacokinetic properties can be incorporated into glycopeptides through semisynthetic modifications.

The pharmacokinetics and distribution of most pharmacological agents have a dramatic influence on their therapeutic utility and duration of action (4, 11). In the case of antimicrobial agents, the physical-chemical properties associated with long durations of action have been examined only in a few cases. The beta-lactam antibiotics with negatively charged side chains in the 3 position, as exemplified by cefonicid (1) and ceftriaxone (2), have long half-lives in serum. Increased serum protein binding decreases the renal clearance of these compounds and extends their half-lives (4, 11). In the case of the sulfonamide antibacterial agents, detailed structure-pharmacokinetic studies have been performed (14). In rats, the rate constant of elimination, renal clearance, and nonrenal clearance of the sulfonamides decrease with increasing lipophilicity and increasing acidity (i.e., increased negative charge). This decrease in renal and nonrenal clearance is partially explained by increased binding to serum. The effects of charge and lipophilicity on the pharmacokinetics of larger molecules have been largely neglected due to the paucity of useful agents with molecular weights in excess of 700. With products of recombinant DNA technology emerging as potential drugs, information on the influence of physical-chemical factors on the disposition of the larger peptidlike molecules is becoming particularly important.

The recent increase in methicillin-resistant, gram-positive bacteria has caused an increase in the use of vancomycin, a glycopeptide antibiotic with a molecular weight of 1,447. We have recently described the discovery, isolation, and characterization of the aridicins, novel glycopeptide antibiotic products of Kibdelosporangium aridum (SK&F AAD216). These antibiotics differ from vancomycin in that they have a lipid acyl side chain and a net negative charge at neutral pHs (10, 15, 16; R. D. Sitrin, G. W. Chan, F. Chapin, A. J. Giovannella, S. F. Grappel, P. W. Jeffs, L. Phillips, K. M. Snader, and L. J. Nisbet, submitted for publication).

The aridicin aglycone is a heptapeptide of highly cross-linked polyphenolic amino acids (R. Sitrin, G. Chan, G. Roberts, C. DeBrosse, L. Mueller, L. Webb, L. Killmer, and P. Jeffs, submitted for publication; Fig. 1). Aridicin A contains one mannose subunit and a novel glycolipid unit, the n-decanoyl amide of 2-amino-2-deoxyglucuronic acid, that are attached through glycoside linkages (10; P. W. Jeffs, L. Mueller, C. DeBrosse, S. Heald, and R. Fisher, submitted for publication). Aridicin B and aridicin C differ from aridicin A in that a C12 or a C18 lipid, respectively, replaces the n-decanoyl moiety. Although these analogs differ from aridicin A by one or two additional methylene units, they are remarkably more lipophilic (16). A series of hydrolysis products were generated to alter the charge and lipophilicity of aridicin A (Sitrin et al., submitted). Pseudoaglycone A is a product of aridicin A that lacks the mannose subunit and, thus, has a similar charge but increased lipophilicity. The mannosyl aglycone lacks the glycolipid subunit, a change which alters both charge and lipophilicity. The aridicin aglycone lacks both the mannose and the glycolipid. The analogs and control compounds that were studied varied in their pIs by four pH units, from 3.8 to 8.5 (16; Sitrin et al., submitted; Table 1). The aridicins and their partial degradation products are active against staphyloococi in vitro (Sitrin et al., submitted), and as also reported for the teicoplanin series (12), the aglycone is the most active molecular species in vitro.

The results of previous prophylaxis experiments in laboratory infection studies indicate that the aridicins and their hydrolysis products have long durations of action (6). In this report, we describe the pharmacokinetics of the aridicins and their hydrolysis products and compare the pharma-
MATERIALS AND METHODS

Animals. Male CD-1 mice (18 to 21 g) were obtained from Charles River Laboratories, Inc. (Wilmington, Mass.) and were quarantined 1 week before use. Mice were fed mouse food pellets from Ralston Purina and were permitted free access to water. Animals were not fed overnight before use.

Antibiotics. Vancomycin and ristocetin were obtained from Sigma Chemical Co. (St. Louis, Mo.). Teicoplanin was obtained by fermentation of Actinoplanes teichomyceticus ATCC 31121. Aridicins A, B, and C were obtained by fermentation of K. aridum ATCC 39323 (16). The partial hydrolysis products pseudoaglycone A, mannosyl aglycone, and aglycone were prepared from aridicin A (Sitrin et al., submitted). All antibiotics were at least 95% pure on high-pressure liquid chromatography (HPLC) analysis (16; Sitrin et al., submitted). Antibiotics were administered in normal saline. The pIs of the antibiotics were determined as described previously (8).

Antibiotic assays. Antibiotic activity in serum was evaluated by disk agar diffusion with penicillin assay agar that was seeded with a Bacillus subtilis ATCC 6633 spore suspension as the indicator. Serum samples were evaluated against standards diluted in mouse serum. Assays were incubated overnight at 30°C. Although the precision of the assay methods was not tested for each antibiotic, the coefficient of variation of this general procedure was 6 to 10%. It is well known that the attached peptide nucleus is required for microbiological activity of glycopeptides (7, 20, 21). Degradation products of the peptide backbone would not be measured as microbiological activity. However, glycopeptides which lost carbohydrates retained microbiological activity. Serum and urine samples were assayed by reversed-phase HPLC as described previously (16; Sitrin et al., submitted) to determine whether aglycone metabolites of glycopeptides were formed in vivo. Samples were prepared for HPLC analysis by adsorption of 1 ml of serum onto a SepPak C18 cartridge (Waters Associates, Inc., Milford, Mass.) and by elution with 20 to 50% acetonitrile in phosphate buffer (pH 3.2).

Pharmacokinetics. Each datum point represents the concentration of antibiotic in the pooled serum from 8 to 10 mice at 15, 30, 60, 120, 180, 240, 300, and 360 min after drug administration. For pseudoaglycone A, additional samples were obtained at 480, 720, and 960 min. The serum concentrations versus time profiles obtained after intravenous administration of glycoproteins were analyzed with the PHARM pharmacokinetic parameter estimation program (5). The terminal half-lives were estimated by unweighted linear regression analysis of the log-transformed data. The total systemic clearance and steady-state volume of distribution were calculated by noncompartmental methods (3). The area under the serum concentration versus time curve was calculated using the linear trapezoidal rule and was extrapolated to time infinity by dividing the last datum point by the terminal rate constant. The concentration of drug at time zero was estimated from the fitted data and used in the calculation of the area under the curve.

RESULTS

The absorption and elimination of vancomycin and the aridicin hydrolysis products mannosyl aglycone and aridicin aglycone were relatively rapid after subcutaneous administration (Fig. 2). However, after subcutaneous administration of aridicins A, B, and C and pseudoaglycone A, the serum concentrations increased with time for 180 to 240 min, indicating slow absorption from the injection site. This slow absorption phase resulted in diminished peak concentrations and increased residence times of the glycopeptides in the body. Thus, subcutaneous administration decreased the acute apparent in vivo potency but extended the duration of action of these glycopeptides. The data obtained after subcutaneous dosing provide information on the antibiotic concentrations in serum under conditions used in mouse protection studies reported previously (6).

Because of the slow absorption after subcutaneous administration, intravenous administration was utilized to obtain detailed pharmacokinetic data and to determine the influence of elimination rate (half-life) on the duration of action of these compounds. The serum concentrations of the aridicins, the aridicin A hydrolysis products, vancomycin, teicoplanin, and ristocetin following intravenous administration are shown in Fig. 3 and 4. Administration of aridicins A, B, and C and pseudoaglycone A (pIs = 3.8) yielded higher, prolonged serum concentrations (half-life range, 226 to 492 min). In contrast, vancomycin and ristocetin (pIs ∼ 8) were rapidly removed, with half-lives of 20 and 63 min, respectively. Teicoplanin, mannosyl aglycone, and aglycone (pIs ∼ 5) had intermediate elimination rates (half-life range, 118 to 155 min). The pharmacokinetic parameters, including total systemic clearance, half-life, and steady-state volume of distribution, calculated from the data obtained from intravenous administration are presented in Table 1. The half-lives of the aridicins and their hydrolysis products were longer than those of vancomycin and ristocetin, and the corre-
sponding clearances and steady-state volumes of distribution were lower. It should be noted that for the aridicins, a considerable portion of the area under the curve was obtained by extrapolation (Table 1).

The recovery of antibacterial activity in urine was determined after subcutaneous and intravenous administration. The urinary recovery of antibiotic activity from 0 to 6 h was, in general, higher after intravenous than subcutaneous administration. In general, as the pI of the antibiotics decreased, the urinary recovery also decreased. For the glycopeptides with long half-lives in serum, the low urinary recovery was in part due to the short collection periods of urine. Nevertheless, these data indicate that the urinary elimination of the antibiotics decreased with decreasing pI.

Because the aglycone derivatives of the aridicins and teicoplanin are known to have higher in vitro potency,
several urine and serum samples were analyzed by HPLC to check for bioconversion (loss of carbohydrates). No measurable levels of aglycone derivatives were observed for the aridicins, teicoplanin, or vancomycin. These results indicate that the microbiological assay measured only parent drug.

**DISCUSSION**

The clearances and half-lives of glycopeptide antibiotics are highly correlated with their pIs. In general, as pi decreases, the clearance and volume of distribution decrease, whereas the half-life increases. The decrease in clearance is much greater than the decrease in volume of distribution, causing a large increase in half-life with decreasing pi. The urinary recovery data for 0 to 6 h (Table 2) indicate that the decrease in total systemic clearance is at least partially due to a reduced rate of renal clearance. Thus, a decrease in the pi appears to result in a reduction in the renal clearance of these glycopeptides.

Changes in the pi alone do not explain all of the observed differences in the pharmacokinetics of these compounds. Reverse-phase HPLC data for aridicins A, B, and C (16; Sitrin et al., submitted) indicate that lipophilicity increases as the length of the lipid chain increases. For these compounds which have identical pIs, the clearance decreased and the half-life increased with increasing lipophilicity.

In some cases, a considerable portion of the area under the curve was obtained by extrapolation (Table 1). This may have caused errors in the estimates of the pharmacokinetic parameters. Therefore, the reported pharmacokinetic parameters for the aridicins should be considered tentative. However, if a second and longer elimination phase was missed due to insufficient sampling, the calculated clearances would be overestimated and the steady-state volumes of distribution and mean residence times would be underestimated. Because the glycopeptides with the longest half-

**TABLE 1. Pharmacokinetic parameters of glycopeptides after intravenous administration**

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Clearance (ml/min/kg)</th>
<th>t1/2 (min)</th>
<th>Vss (ml/kg)</th>
<th>% AUC extrapolated</th>
<th>pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>16.36</td>
<td>20</td>
<td>419</td>
<td>1.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>8.18</td>
<td>62</td>
<td>755</td>
<td>9.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Mannosyl aglycone</td>
<td>2.91</td>
<td>135</td>
<td>498</td>
<td>1.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Aridicin aglycone</td>
<td>3.98</td>
<td>118</td>
<td>695</td>
<td>7.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.72</td>
<td>155</td>
<td>152</td>
<td>20</td>
<td>5.1</td>
</tr>
<tr>
<td>Aridicin A</td>
<td>0.66</td>
<td>226</td>
<td>222</td>
<td>38</td>
<td>3.8</td>
</tr>
<tr>
<td>Aridicin B</td>
<td>0.56</td>
<td>232</td>
<td>185</td>
<td>33</td>
<td>3.8</td>
</tr>
<tr>
<td>Aridicin C</td>
<td>0.35</td>
<td>277</td>
<td>136</td>
<td>39</td>
<td>3.8</td>
</tr>
<tr>
<td>Pseudoaglycone A</td>
<td>0.18</td>
<td>492</td>
<td>131</td>
<td>27</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* t1/2, half-life; Vss, steady-state volume of distribution; % AUC extrapolated, percentage of area under the curve from the last time point to time infinity.

**TABLE 2. Urinary recovery of glycopeptides after subcutaneous and intravenous administration**

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Urinary recovery from 0 to 6 h (% of dose) for the following routes of administration:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>68</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>ND*</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>11</td>
</tr>
<tr>
<td>Mannosyl aglycone</td>
<td>20</td>
</tr>
<tr>
<td>Aridicin aglycone</td>
<td>6</td>
</tr>
<tr>
<td>Aridicin A</td>
<td>12</td>
</tr>
<tr>
<td>Aridicin B</td>
<td>9</td>
</tr>
<tr>
<td>Aridicin C</td>
<td>3</td>
</tr>
<tr>
<td>Pseudoaglycone A</td>
<td>1</td>
</tr>
</tbody>
</table>

* ND, Not determined.
lives and lowest clearances had the greatest portion of the area under the curve extrapolated, this possible error may have artificially decreased the differences between the pharmacokinetics of these glycopeptides. Nevertheless, the overall correlation of physical-chemical and pharmacokinetic parameters with these glycopeptides was not altered by this possible error.

Quantitative structure-pharmacokinetic relationships in animals and humans have been reported for only a few classes of drugs (9, 13, 14, 17). In general, lipophilic-hydrophilic and electronic properties are far more important than steric characteristics in governing the overall pharmacokinetic behavior of drugs. It is well known that positively charged drugs display low binding to serum and negatively charged drugs exhibit high binding to serum (4, 11). It is also known that increases in drug lipophilicity generally are associated with increases in serum binding (4, 11). Increased binding to serum decreases the fraction of drug available for drug elimination (decreased renal filtration) and can thus decrease drug clearance. The effect of charge on drug distribution (volume of distribution) depends on the relative effect of charge on drug binding to serum and tissue proteins. Because vancomycin is renally cleared by glomerular filtration and the urinary recovery of the aridicins is low, the observed low renal clearance of the aridicins is consistent with increased binding to serum with decreased 

\[ \text{pl.} \] 

Increased binding to serum could also explain the trend toward a smaller volume of distribution with decreasing 

\[ \text{pl.} \] 

Differences in the pharmacokinetics of glycopeptides with similar 

\[ \text{pl.} \] 

may be explained by differences in lipophilicity. As the lipophilicity of the aridicins (A < B < C) increases, clearance decreases and half-life increases. This hypothesis is consistent with previous findings concerning the correlation of physical-chemical properties and drug disposition of small organic molecules (4, 11). Studies in which the binding of an extensive series of glycopeptides to serum are being examined are in progress to examine this hypothesis.

Results of this study demonstrate that the aridicins and their partial hydrolysis products have much longer half-lives than vancomycin and ristocetin. The aridicins, but not their hydrolysis products, have half-lives longer than that of teicoplanin. The fact that teicoplanin has a long half-life in humans and animals (18, 19) suggests that the results of these studies in mice may be useful in predicting the pharmacokinetics in humans. Thus, the aridicins and their hydrolys products may have long half-lives in humans. The strong correlation between pharmacokinetics and physical-chemical properties, with serum protein binding being the likely basis of this correlation, indicates that desirable pharmacokinetic properties can be incorporated into glycopeptides through semisynthetic modifications. These findings are the first report of the correlation of physical-chemical properties with the disposition of large peptidilike molecules.

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**LITERATURE CITED**
