Vancomycin in Rabbits: Pharmacokinetics, Extravascular Diffusion, Renal Excretion and Interactions with Furosemide

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ABSTRACT

The pharmacokinetics, renal excretion, protein binding and extravascular diffusion of vancomycin in rabbits were studied. The effects of furosemide on these different parameters also were investigated. We observed a $T_{1/2}$ of 55 min and protein binding of 65% as determined in vitro by equilibrium dialysis. Vancomycin appeared to be secreted by renal tubules (fractional excretion: 177 ± 44%). In vitro, furosemide (5 μg/ml) slightly decreased the vancomycin protein binding (from 65 to 57%). Furosemide significantly increased the renal excretion of vancomycin, through a tubular process without any effect on the filtered load. Vancomycin appeared slowly and at low concentrations in the extravascular fluid. The extravascular concentrations were higher when the antibiotic was administered by a 6-hr continuous infusion than when given by a 20-min infusion of the same dose. Our results suggested that the in vivo antibacterial effect of vancomycin could be enhanced by prolonged infusion. Also, it was demonstrated that furosemide has only a small effect on the kinetics of vancomycin.

The emergence of staphylococci resistant to multiple antibiotics generated a new interest in vancomycin. In addition, vancomycin has proved valuable for treating severe infections, especially endocarditis, in patients with β-lactam antibiotic allergy (Cook and Farrar, 1978; Manuel et al., 1980). Despite the renewed clinical interest in vancomycin, there are few studies of its pharmacokinetic properties and mechanisms of urinary excretion (Nielsen et al., 1975; Krogstad et al., 1980; Moellering et al., 1981). In addition, the extent of serum protein binding of this antibiotic remains controversial (Lindholm and Murray, 1966; Krogstad et al., 1980).

During treatment with vancomycin of severe infections, furosemide is sometimes administered at high doses when a rapid onset of diuresis is desired. To our knowledge, nothing is known about the interactions between furosemide and vancomycin.

The purpose of this paper was to assess the kinetics of i.v. vancomycin and the mechanisms of its renal excretion, and to describe the effects of furosemide, a highly protein-bound diuretic, on the disposition of vancomycin. In rabbits, three aspects of the interaction were studied: serum protein binding in vitro, extravascular diffusion on a previously described tissue cage model (Carbon et al., 1977) and urinary excretion.

Materials and Methods

Animal model. The investigations were carried out in female rabbits (Fauve de Bourgogne; weight range 2.5-3.0 kg). Extravascular fluid (tissue cage fluid) was obtained from s.c. tissue cages, as previously described (Carbon et al., 1977).

Kinetic study. Four rabbits received a single i.v. bolus injection of vancomycin (15 mg/kg). Blood samples were collected 3, 5, 10, 15, 30, 60, 120, 240, 360 and 480 min after the injection. The parameters of the two-compartment open system kinetic model were calculated by iterative fitting and the computation procedures were performed using the SAAM 25 program (Berman and Weiss, 1971). Protein binding study. Protein binding was investigated by equilibrium dialysis using a Dianorm system (Diachema AG, Rüschlikon, Switzerland) with 2-ml cells and cellulose dialysis membranes (Union Carbide Corporation, Chicago, IL) during 4 hr at 37°C, in 0.15 M phosphate buffer (pH 7.4). Antibiotic concentrations were measured on each side of the dialysis membrane. The stability of the molecule was verified after a 4-hr incubation at 37°C in buffer and serum.

Extravascular diffusion study. This study was divided in two parts.

1. Single 20-min i.v. infusions of vancomycin (15 mg/kg) were administered alone or combined with furosemide (3 mg/kg) injected i.m. into six rabbits. Blood and tissue cage fluid were collected for antibiotic assays at times indicated in the tables.

2. Six-hour i.v. infusions of vancomycin (15 mg/kg) were administered to six rabbits. Blood and tissue cage fluids were collected for antibiotic assays at the times indicated in the tables. CSF was withdrawn by suboccipital puncture, 2 and 6 hr after the beginning of the infusion.

Urinary excretion study. The renal handling of vancomycin and the effect of furosemide on the urinary excretion of the antibiotic were

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ABBREVIATIONS: CSF, cerebrospinal fluid.
studied in six rabbits. The animals were anesthetized briefly with ketamine hydrochloride administered by i.m. injection (10 mg/kg). Two catheters were inserted into the femoral veins for infusion and sampling. Urine was collected from both ureters catheterized after a suprapubic incision. The wounds were carefully closed around the catheters with surgical silk after local infiltration with lidocaine and then packed with gauze treated in saline. Two hours after ketamine injection, a continuous infusion of saline was started at a rate of 0.5 ml/kg/min. Vancomycin (15 mg/kg/hr) was infused concomitantly with 

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iothalamate (0.1 μCi/min) (Amersham, Versailles, France) over a period of 2 hr for equilibration. After two control periods of 15 min duration each, furosemide was injected i.v. (3 mg/kg) followed by two experimental periods of 15 min and two of 30 min duration each. Blood samples for antibiotic, iothalamate and sodium assays were collected at the end of each control and experimental clearance period. Urines were obtained throughout the six periods. For each period, we calculated 1) the vancomycin filtered load, 2) the vancomycin excreted/infused ratio and 3) the absolute rate of net vancomycin tubular secretion, as the rate of urinary excretion of vancomycin minus the rate of glomerular filtration of vancomycin; the latter was calculated as the product of unbound vancomycin blood concentration and glomerular filtration rate, i.e., iothalamate renal clearance. We also calculated the absolute renal clearance of vancomycin, uncorrected for protein binding. The vancomycin and sodium fractional excretions were also determined. The control values were established from 12 determinations, two control periods in six rabbits.

Assays. Blood samples were first allowed to clot and then centrifuged at 3,500 rpm for 15 min. Serum, “tissue cage fluid” and urine were stored at −30°C.

Antibiotic assays. Standards for the assay of serum samples were prepared in normal rabbit serum. Standards for the assays of tissue cage fluid samples were prepared with rabbit serum diluted 3-fold in 0.15 M phosphate buffer (pH 7.4) to reduce the level of total protein to that observed in tissue cage fluid (Carbon et al., 1977). Standard curves obtained in these conditions correlated well with those made in tissue cage fluid. Standards for the assay of urine samples were prepared in a 0.15 M phosphate buffer (pH 7.4).

Each serum, tissue cage fluid and urine sample was studied in duplicate. Concentrations of antibodies were determined by diffusion in nutrient agar according to the method of Crossley et al. (1980) with Bacillus cereus (Institut Pasteur 5832, Paris) as the test organism. Results were within 10% of the known value for concentrations less than 25 μg/ml and reproducible between 0.5 and 25 μg/ml. Samples for vancomycin determinations were diluted when necessary.

### Results

**Kinetic study.** The pharmacokinetic data are as follows: volume of central compartment: 164 ± 15 ml/kg; volume of peripheral compartment: 395 ± 30 ml/kg; \( T_{1/2} \) of the distribution phase (\( T_{1/2}^{\alpha-β} \)): 3.65 ± 0.23 min; \( T_{1/2} \) of the elimination phase (\( T_{1/2}^{β} \)): 54.13 ± 3.04 min; volume of distribution [\( V_{D(AUCC)} \)] 781 ± 60 ml/kg; and total body clearance: 4.1 ± 0.6 ml/kg/min.

**Protein binding study.** The percentage of serum protein binding of vancomycin was 65 ± 5% when this drug was studied alone at a concentration of 20 μg/ml. Furosemide weakly reduced the extent of binding of vancomycin. Values of 62 ± 3% and 57 ± 4% were measured with furosemide concentrations of 1 and 5 μg/ml, respectively. The latter percentage only was significantly different from the control value (P < .01).

**Extravascular diffusion study 1.** Serum levels obtained after a 20-min i.v. infusion of vancomycin administered alone or combined with furosemide are given in table 1. The extravascular fluid concentrations of vancomycin are presented in table 2. The antibiotic was not detectable at 30 min when administered alone, or at 30 and 60 min when administered with furosemide.

**Extravascular diffusion study 2.** Serum, extravascular fluid and CSF concentrations of vancomycin obtained after 6 hr of i.v. infusion of the antibiotic are reported in table 3. At the end of the infusion, there was no equilibration between

### Table 1

**Extravascular diffusion study (1): serum concentrations of vancomycin**

<table>
<thead>
<tr>
<th>Time</th>
<th>20 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>V alone</td>
<td>34.0 ± 7.9</td>
<td>25.7 ± 7.2</td>
<td>7.5 ± 1.1</td>
<td>2.4 ± 0.6</td>
<td>1.0 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>V + F</td>
<td>35.7 ± 4.0</td>
<td>22.0 ± 2.2</td>
<td>10.2 ± 2.4</td>
<td>3.4 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2

**Extravascular diffusion study (1): extravascular fluid concentrations of vancomycin**

<table>
<thead>
<tr>
<th>Time</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
<th>480 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>V alone</td>
<td>0.93 ± 0.08</td>
<td>1.12 ± 0.20</td>
<td>1.27 ± 0.27</td>
<td>1.15 ± 0.14</td>
<td>1.0 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>V + F</td>
<td>0.92 ± 0.16</td>
<td>1.22 ± 0.26</td>
<td>1.18 ± 0.29</td>
<td>1.1 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

**Extravascular diffusion study (2): serum, extravascular fluid and CSF concentrations of vancomycin**

<table>
<thead>
<tr>
<th>Time</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
<th>480 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>10.25 ± 2.42</td>
<td>10.88 ± 2.39</td>
<td>11.75 ± 2.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extravascular fluid</td>
<td>1.60 ± 0.18</td>
<td>2.90 ± 0.8</td>
<td>3.95 ± 0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4

Urinary excretion of vancomycin (V) and effects of furosemide

V (15 mg/kg/hr) was continuously infused with 72HCO3 in saline (0.5 ml/kg/min). Two hours were needed for equilibration. Two control periods of 15 min duration each were then observed. Control period values reported in the table represent mean ± S.D. of these two control periods. At this time, furosemide was injected i.v. (3 mg/kg). Four experimental periods were then observed (15 min for periods 1 and 2 and 30 min for periods 3 and 4). Each value represents mean ± S.D. obtained from six animals.

<table>
<thead>
<tr>
<th>Control Period</th>
<th>Experimental Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>UFR</strong></td>
<td>0.32 ± 0.31</td>
</tr>
<tr>
<td><strong>GFR</strong></td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td><strong>Total serum concentration</strong></td>
<td>35 ± 4</td>
</tr>
<tr>
<td><strong>Filtered load</strong></td>
<td>63 ± 7</td>
</tr>
<tr>
<td><strong>Absolute rate net tubular secretion</strong></td>
<td>49 ± 33</td>
</tr>
<tr>
<td><strong>Fractional excretion (%)</strong></td>
<td>177 ± 44</td>
</tr>
<tr>
<td><strong>V excreted/infused (%)</strong></td>
<td>48 ± 16</td>
</tr>
<tr>
<td><strong>V absolute renal clearance (%)</strong></td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td><strong>Sodium fractional excretion (%)</strong></td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

* Urinary flow rate (milliliters per minute).
* Glomerular filtration rate (milliliters per kilogram per minute).
* Micrograms per milliliter.
* Micrograms per minute.
* Milliliters per kilogram per minute.
* Significantly different from the control value (P < .01).

Urinary excretion study. The results obtained are reported in table 4. The fractional excretion of vancomycin during the control period was 177 ± 44%. After furosemide injection, the glomerular filtration rate decreased slightly but significantly during the periods 2, 3 and 4 as compared to the control value. The diuretic effect was marked, as evidenced by increased urinary flow rate and increased sodium fractional excretion. The fractional excretion and the absolute rate of net tubular secretion of vancomycin were significantly increased. The percentage of excreted-infused vancomycin significantly increased after furosemide during the first experimental period. Total vancomycin serum levels remained identical throughout the study. The renal clearance of vancomycin (uncorrected for binding) was 3.2 ± 0.9 ml/kg/min in the control period. A significant increase was observed in the first experimental period.

Discussion

Four aspects of our results deserve further comment.

Protein binding. The serum protein binding of vancomycin measured in our study in rabbits (65%) was similar to that obtained in man (55%) by Krogstad et al. (1980), but was quite different from that suggested in another study (less than 10%) (Lindholm and Murray, 1966). This extent of binding could explain the slow diffusion of vancomycin into the extravascular fluid. However, as suggested in previous studies (Carbon et al., 1977; Craig and Welling, 1977), the relatively low extravascular concentrations of vancomycin probably are related to one or more factors in addition to the extent of protein binding. In vitro, the binding of vancomycin to serum proteins decreased slightly and was significant only in the presence of the higher concentration (5 μg/ml) of furosemide. This pattern is quite different from that observed with cefazolin, the protein binding of which was reduced even by very low concentrations of furosemide (0.5 μg/ml) (Carbon et al., 1980). In a previous study, we have measured plasma levels of furosemide higher than 5 μg/ml only up to 15 min after i.v. bolus injection of 3 mg/kg (Carbon et al., 1980). It is thus conceivable that the displacing effect of furosemide on the binding of vancomycin was of short duration after i.v. injection and weak or nil after i.m. injection of the same dose. This could explain the fact that when administered concomitantly with furosemide, vancomycin did not appear more rapidly in the extravascular fluid. In contrast with what was observed with cefazolin in the same conditions, vancomycin appeared later when injected with furosemide than when administered alone. This could be related to the prominent effect of furosemide on the renal excretion of vancomycin.

Kinetics and renal excretion. Our study suggested that the elimination T1/2 was shorter in rabbit (less than 1 hr) than in man (4.7–11.2 hr) (Krogstad et al., 1980). The vancomycin body clearance was greater in rabbits (4.1 ml/min/kg) than in man (1.09–1.37 ml/min/kg) (Krogstad et al., 1980). It should be noted that the glomerular filtration rate observed in our study (5.2 ml/kg/min) was greater than that found in man (1.31–1.67 ml/kg/min). The comparison of the total body clearance of vancomycin to its renal clearance (3.2 ± 0.9 ml/kg/min) suggested that the antibiotic is cleared to a small extent by non-renal routes in rabbits. There was no evidence that vancomycin is cleared by extrarenal routes in man (Kirby and Divelbiss, 1957; Lee et al., 1957). We calculated the fractional excretion of vancomycin by using the serum concentration of free drug. Under these conditions, we found during the control periods a fractional excretion of vancomycin of 177 ± 44%, indicating that this antibiotic was secreted by the tubules. These results were different from those obtained by Krogstad et al. (1980), who described a fractional excretion of vancomycin from 59 to 75% in man. These authors apparently made their calculations from the blood concentrations of the total drug. On the basis of our findings and with their percentage of protein binding (55%), their values once recalculated are 131 to 167%, suggesting a net secretion of the drug in man in the same range as in rabbits. Therefore, the differences observed between the elimination T1/2 values found by these authors and the values obtained in our study appeared essentially related to the higher glomerular filtration rate observed in rabbits. However, these data must be interpreted according to the fact that physiological function per unit of animal weight decreases as size increases (Dedrick, 1973).

Effects of furosemide on the urinary excretion of vancomycin. In order to determine the absolute rate of net van-
of treatment due to higher daily doses and possible enhanced extravascular levels. However, in the extravascular levels obtained after a 20-min i.v. infusion of the vancomycin concentrations measured in extravascular fluid after 20-min i.v. infusion or 6-hr i.v. infusion of the same total therapeutic dose (15 mg/kg) demonstrated the influence of the mode of vancomycin administration on the extravascular distribution of this drug. The extravascular levels obtained after a 6-hr i.v. infusion were higher than those measured after a 20-min i.v. infusion. This phenomenon seems specific to the antibiotic used. Thus, the present results are quite different from those obtained with cefalothin in a previous study (Carbon et al., 1978).

The steady-state equilibrium between serum and extravascular fluid was not reached, after a period of infusion equivalent to 6.5 T1/2 β of vancomycin, even when considering the levels of free antibiotic in blood (55% of the total serum level) and the reduced binding of antibiotics in extravascular fluid with low protein content (Peterson and Gerding, 1978). Prolonged i.v. infusions of methicillin have previously been studied in rabbits (Gengo and Schentag, 1981). In this latter study, the steady-state equilibrium between serum and tissue cage fluid levels was obtained only 6 hr (i.e., 12 T1/2 β) after the beginning of a continuous infusion of high doses of the drug (up to 90 mg/kg/hr). The interpretation of these data remains identical when the low extent of methicillin binding to serum proteins (17%) is taken into account. Our results with vancomycin suggested that higher extravascular fluid concentrations were achieved after a 6-hr infusion than after a 20-min infusion. The enhanced concentrations obtained with the former mode of injection have to be considered in the treatment of severe infections due to microorganisms with borderline sensitivity to vancomycin. Also, the progressive accumulation noted in extravascular areas after repetitive doses has to be taken into account (Carbon et al., 1977; Peterson et al., 1981; Gengo and Schentag, 1981). It is possible that a continuous infusion of vancomycin equivalent to 15 mg/kg/6 hr (i.e., 60 mg/kg/day) could ameliorate the extravascular levels. However, in this hypothesis, a greater cost of treatment due to higher daily doses and possible enhanced ototoxicity have to be taken into consideration.

Our results were obtained in rabbits after a single injection of both vancomycin and furosemide and did not allow any conclusion concerning what could happen with repetitive doses in humans. However, these results seem to indicate that this drug weakly diffuses into the extravascular fluid. The prolonged i.v. infusion of vancomycin is likely to induce a more important penetration of this antibiotic in extravascular fluids. The interaction of furosemide on the disposition of vancomycin seems to be weak and does not suggest any evidence of potentiation by the diuretic of the antibiotic toxicity, at least through pharmacokinetic interactions.

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References

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