In-vivo activity and pharmacodynamics of cefotaxime in combination with vancomycin in fibrin clots infected with highly penicillin-resistant Streptococcus pneumoniae

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We studied the antipneumococcal efficacy of cefotaxime and vancomycin alone and a combination of cefotaxime with various dosages of vancomycin in the treatment of prolonged (48 h) experimental fibrin clot infections in rabbits. A clinical pneumococcal strain for which MICs were 2, 0.5 and 0.5 mg/L of penicillin, cefotaxime and vancomycin respectively, was used in this study. Cefotaxime was given iv at a fixed dose of 50 mg/kg and vancomycin iv at 1, 2.5, 5, 10 or 20 mg/kg. Maximal concentrations in clots were (mean ± S.D.): 2.1 ± 0.9, 1.1 ± 0.4, 1.9 ± 1, 2.3 ± 1.5, 3.6 ± 0.4 and 4 ± 0.3 mg/g, respectively. The mean half-lives of elimination from clots were 2.2 h for cefotaxime and 7 h for vancomycin. We observed the highest bacterial reductions for the highest doses of vancomycin with or without cefotaxime. The combination of intermediate doses of vancomycin with cefotaxime led to higher antibacterial effects than either monotherapy. The low dose of vancomycin gave no significant additional effect compared with cefotaxime alone. The times of regrowth were similar for cefotaxime and cefotaxime-vancomycin 1, and also for vancomycin 10 and vancomycin 20 with or without cefotaxime but were significantly delayed for the combination cefotaxime-vancomycin 2.5 and cefotaxime-vancomycin 5 as compared with vancomycin 2.5 and vancomycin 5. By using a multivariate analysis, we demonstrated that the most important parameters were Cmax (r = 0.43) and AUC (r = 0.58) for cefotaxime alone and Cmax (r = 0.70) for vancomycin alone; none of the tested parameters was found to be significantly correlated with the efficacy of the combinations of cefotaxime and vancomycin. From these findings, and under the experimental conditions used (i.e., relatively low concentrations of cefotaxime), we demonstrated that the in-vivo antibacterial efficacy of the combination of cefotaxime and vancomycin was higher than each monotherapy when the local concentrations of vancomycin were at least 1.9 mg/L.

Introduction

Despite the availability of potent new antimicrobial agents, infections caused by Streptococcus pneumoniae continue to represent a significant cause of mortality and morbidity in humans (Appelbaum, 1992; Bouza & Munoz, 1995; Klugman & Friedland, 1995). A dramatic increase in the incidence of pneumococcal strains with resistance to penicillin and a range of other antimicrobial agents has been observed over the past two decades (Zighelboim & Tomasz, 1980; Buu-Hoi, Goldstein, & Acar, 1988; Appelbaum, 1992; Chesney, 1992; Friedland & Klugman, 1992b; Garcia-Leoni et al.,
In this era of increasing resistance and since poor responses to therapy and increased mortality resulting from pneumococcal infections have been seen (Radetsky et al., 1981; Caputo et al., 1983; Viladrich et al., 1988; Friedland & Klugman, 1991; Friedland & Istre, 1992; Friedland & Klugman, 1992a; Mac Dougal et al., 1992; Caputo, Appelbaum, & Liu, 1993; Hulbert, Larsen, & Chandrasoma, 1993; Bouza & Munoz, 1995; Klugman & Friedland, 1995), it seems reasonable to re-examine all modalities potentially available for treating pneumococcal infections. The optimal therapeutic approach towards such cases is not known (Tan et al., 1994; Paris, Ramilo, & McCracken, 1995), but expanded-spectrum cephalosporins such as cefotaxime or ceftriaxone are the most commonly recommended (Klugman & Friedland, 1995). However, failures have been reported with monotherapy with these compounds (Bradley & Connor, 1991; Sloas et al., 1992; Friedland, Shelton, & Paris, 1993b; John, 1994; Olivier et al., 1994).

Vancomycin is not affected by the intrinsic resistance of pneumococci to β-lactam agents (Linares et al., 1984; Splangler, Jacobs & Appelbaum, 1992; Barakett et al., 1993; Geslin et al., 1994b; Pankuch, Jacobs, & Appelbaum, 1994; Appelbaum, 1995) and its use has been suggested as therapy for highly penicillin resistant pneumococcal infections including meningitis (Tunkel, Wilsapelwey & Scheld, 1990; Viladrich et al., 1991; Longuet et al., 1993; Bouza & Munoz, 1995; Klugman & Friedland, 1995). In these situations, vancomycin is considered as a second line therapy or is added to β-lactam antibiotics (Astruc, 1994; McCracken, 1994, Tan et al., 1994; Lister, 1995).

The antibacterial effect on highly penicillin-resistant pneumococcal infections of a combination of a β-lactam and vancomycin is variable depending upon the experimental conditions: often indifferent in vitro (Doit et al., 1992; Cassinat & Nicolas, 1994) or synergistic in vivo (Friedland et al., 1993a; Friedland et al., 1994).

Since these observations are somewhat unclear in a therapeutic perspective, in this study we investigated the conditions of an increased in-vivo antibacterial effect of the combination cefotaxime with vancomycin on an infection caused by a highly penicillin resistant pneumococcal strain. By using a prolonged (48 h) fibrin clot model in rabbits, we have tested the intrinsic therapeutic efficacy of cefotaxime and vancomycin alone and the combination cefotaxime-vancomycin with various dosages of vancomycin.

Materials and methods

Bacterial strain and susceptibility studies

The pneumococcal strain used (No 9093 serotype 9 v, kindly provided by the Centre de Référence des Pneumocoques, Dr Geslin) was obtained from the cerebrospinal fluid (CSF) of a patient with meningitis. MICs of the study drugs were determined by the standard tube dilution technique, in which an inoculum of \(5 \times 10^4\) cfu and Mueller-Hinton broth supplemented with 5% horse blood, 0.25 mL of MgCl\(_2\) and 0.5 mL CaCl\(_2\) were used. The MIC was defined as the lowest concentration of drug that was required to inhibit visible growth. Since we used the fibrin clot model in rabbits, we checked for susceptibility of this pneumococcal strain in fibrin. The same dilution technique was used but the broth was a 2.5% solution of sterile bovine fibrinogen in brain heart broth. As shown previously (Chavanet et al., 1995), MICs were the same
with both methods and were 2, 0.5 and 0.5 mg/L for penicillin, cefotaxime, and vancomycin, respectively.

**Experimental model**

Infected fibrin clots were prepared as follows: a 2 mL sterile solution of 2.5% bovine fibrinogen was distributed into test tubes (13 x 100 mm) and infected with 100 µL of a 5 h culture of the strain (inoculum $10^6$ cfu/mL). Human thrombin (Laboratoire Organon, Fresnes, France) was added to each tube, and the tubes incubated at 37°C for 1 h. The resulting clots were then gently removed, and immediately inserted subcutaneously into rabbits.

We used a modified technique of the subcutaneous fibrin clot model in rabbits (Bergeron, Robert & Beauchamp, 1993) in order to obtain a prolonged observation over 48 h. New-Zealand white male rabbits (2-3 kg) were anaesthetized with ketamine and xylazine, and silastic central venous catheters were placed by using a sterile operative technique as described previously (Walsh, Bacher & Pizzo, 1988) to provide for continued nontraumatic venous access. One flank was shaved, swabbed with iodine and alcohol and then the skin was anaesthetized with 2% lidocaine before making a 4 cm incision. On the day of experiment, eight infected clots were placed in the subcutaneous pocket. Autoclips were applied to close the incision.

**Antibiotic regimens**

Antibiotics were injected into rabbits immediately after clots were inserted. Each antibiotic was infused as a 30 second bolus. Rabbits were randomised to receive: saline as a control; cefotaxime 50 mg/kg or vancomycin (solution at 5 mg/mL) from 1 mg/kg to 20 mg/kg. With combinations, cefotaxime was given at the same dose.

**Determination of drug concentration and pharmacokinetics**

Each clot was weighed and homogenized in a 2.5% solution of trypsin (Laboratoire Eurobio, Les Ulys, France), the volume of which was equal in weight to the clot. Trypsin had no effect on antibiotic activity and did not influence the bacterial count. Dissolved clots and serum samples were assayed for antibiotic concentrations.

Concentrations of cefotaxime were measured by high-performance liquid chromatography as previously described (Patel et al., 1981; Kazmierczak et al., 1982) after an acetonitrile—dichloromethane extraction. The detection limit was 0.082 mg/L in serum and 0.095 mg/L in trypsinized fibrin clots; the intra- and inter-assay variations of this procedure were <6%. Standards of serum samples were diluted in 100% normal rabbit serum, whereas standards of fibrin clots samples were diluted in trypsinized fibrin clots. Concentrations of vancomycin were assayed by fluorescence-polarization immunoassay (Abbot Diagnostic, Rungis, France). Serum samples were obtained at the following times: 0, 5 min, 1, 3, 6, 9, 12, 24, 36 and 48 h. Fibrin clot samples were obtained at 0, 1, 3, 6, 9, 12, 24, 36 and 48 h. The area under the concentration-versus-time curve (AUC) was obtained by the method of successive trapezoidal approximation (Bergeron et al., 1993); and the AUC observed above the MIC level was also obtained (AUCI). The half-lives of elimination of the antibiotics in serum and clots were calculated by the least-squares method. The maximum concentration was considered as the concentration 5 min after the end of the
infusion in serum and the highest observed concentration in clots. Time above MIC in fibrin clots \((T > \text{MIC})\) was calculated from each individual concentration-versus-time curve. Concentration at time of regrowth was the concentration observed in clots at time of bacterial regrowth (see below). This concentration defined the critical concentration below which the bacteria grew in this in-vivo model.

In vivo efficacy

The efficacy of the various regimens was evaluated by the analysis of the bacterial content of infected clots at each interval of time using appropriate dilutions of trypsinized clots which were inoculated on blood agar, followed by incubation at 37°C for 24 h (log cfu/g of clot). The method was sufficiently sensitive to detect \(\geq 10\) cfu/g.

Time of regrowth was defined for each individual experiment as that time when the rate of bacterial growth became positive. Also, the reduction of bacterial content in fibrin clots between time 0 and time of regrowth \((\Delta \log_{10} \text{cfu/g at time of regrowth})\) was calculated for each individual experiment; this parameter defined the best antibacterial effect for each individual experiment. Following the bacterial curve after this latter point, the rate of regrowth was calculated and expressed as log cfu/g per hour.

Statistical analysis

The required number of in-vivo experiments was determined with a type I error of 5% and type II error of 20% for a two-sided test and a difference of one log cfu at 24 h; therefore, results were expressed as the mean (± standard deviation) of at least five experiments for each antibiotic regimen. Statistics were performed by one-way analysis of variance (ANOVA) and the following tests were used for specific comparisons: the protected least significant difference test of Fisher was used to test the difference of results between each pair of regimens; the contrasts method was used to test difference of the results between specific groups of regimens (i.e. with or without either cefotaxime or vancomycin); the least significant range using the Newman-Keuls' test (with \(\alpha = 0.05\)) was used to compare treatments all together. Univariate coefficient ratio estimates were computed with exact methods. In order to standardise the comparison of all the regimens, the best antibacterial effect of each individual experiment \((\Delta \log_{10} \text{cfu/g})\) at time of regrowth, was considered. Statistical significance was accepted if \(P\) value was less than 0.05.

Results

In vitro killing curves

As shown in Figure 1, each antibiotic was tested as fractions and multiples of the MIC. All the combinations of vancomycin plus cefotaxime were associated with higher antibacterial effect than each drug alone (Figure 1(c); \(P < 0.01\)).
Combined effects of cefotaxime and vancomycin

Pharmacokinetics study

Serum

Pharmacokinetic data for each drug were obtained from serum studies in at least five animals (Table I and Figure 2(a)). The highest mean concentration in serum was observed 5 min after the bolus of cefotaxime and vancomycin. The peak of vancomycin significantly increased with the dose.

The respective half-life of cefotaxime was significantly shorter than that of vancomycin; no differences were observed within the vancomycin regimens. Consequently, the areas under the curves of concentrations versus time were the lowest for the lowest doses of vancomycin.

Fibrin clots: maximum concentration, time of peak, half-life of elimination

Pharmacokinetic data in fibrin clots are shown in Table I and Figure 2(b). Maximal concentration of cefotaxime was on average 2.1 mg/L, obtained between the first and second hours.
Table I. Pharmacokinetics in serum and infected fibrin clots of cefotaxime and vancomycin (means ± s.d.)

<table>
<thead>
<tr>
<th>Regimen</th>
<th>C_{\text{max}} (mg/L)</th>
<th>T_{\text{max}} (h)</th>
<th>Half-life (h)</th>
<th>AUC fibrin clots (mg.h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>146 ± 17</td>
<td>5</td>
<td>2.2 ± 1.6</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>10.5 ± 5</td>
<td>5</td>
<td>3.6 ± 1</td>
<td>18.5 ± 7</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>38 ± 11</td>
<td>5</td>
<td>2.4 ± 1</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>50 ± 14</td>
<td>5</td>
<td>2.4 ± 1</td>
<td>29 ± 20</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>71 ± 18</td>
<td>5</td>
<td>1.9 ± 1</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>105 ± 34</td>
<td>5</td>
<td>1.6 ± 1</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>1.1 ± 0.4</td>
<td>5</td>
<td>6.3 ± 2</td>
<td>35.3 ± 10</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>1.9 ± 0.4</td>
<td>5</td>
<td>2.0 ± 0.8</td>
<td>29 ± 20</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>2.3 ± 1.5</td>
<td>5</td>
<td>1.9 ± 1</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>3.6 ± 0.4</td>
<td>5</td>
<td>1.6 ± 1</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>4 ± 0.3</td>
<td>5</td>
<td>1.6 ± 1</td>
<td>61 ± 11</td>
</tr>
</tbody>
</table>

*ANOVA, P ≤ 0.001 for the regimens containing vancomycin.

*ANOVA, P = 0.0027 for the regimens containing vancomycin.

*ANOVA, P = 0.08 for the regimens containing vancomycin.
third hour following the intravenous dose. As expected, the maximal concentration of vancomycin increased with an increase in dose from 1.1 to 4 mg/L. Although the range of times for vancomycin peaks was rather large, the mean time was approximately 3 h after the injection.

Figure 2. Serum (a) and fibrin clot (b) concentrations of cefotaxime and vancomycin (means of at least five experiments in rabbits). O, Cefotaxime iv 50 mg/kg at 0 h; A, vancomycin iv at 0 h; 1 mg/kg; ▲, 2.5 mg/kg; △, 5 mg/kg; ■, 10 mg/kg; ●, 20 mg/kg.
The half-life of cefotaxime was significantly shorter than that of vancomycin (on average 2.2 vs 7 h, respectively); no differences were observed within the vancomycin regimens. Consequently, the areas under the curves of concentrations versus time significantly increased with the dose of vancomycin.

No pharmacokinetic interactions between cefotaxime and vancomycin were observed.

**Fibrin clots: time above MIC, in-vivo critical concentration**

The time during which the concentration was above the MIC for this strain was 9.2 ± 1.4 h for the cefotaxime regimen, which was significantly lower than that observed with each vancomycin regimen (Table II). The times above MIC for the vancomycin regimens seemed to increase with the dose; however the range of variation was quite large and no significant differences were found.

The in-vivo critical concentration was defined as the concentration observed in clots at time of bacterial regrowth. These concentrations were 0.77 ± 0.47 mg/L for cefotaxime and between 0.47 ± 0.56 mg/L and 1.2 ± 0.81 mg/L for vancomycin. No statistically significant differences were detected for the vancomycin monotherapies.

In the combinations, the concentrations at bacterial regrowth for cefotaxime were significantly lower than that observed with cefotaxime alone (on average 0.16 mg/L vs 0.77, \( P < 0.001 \)). No such differences were found for vancomycin.

**Bacteriologic effect**

**Monotherapies**

In the particular experimental conditions used in this study (ie-relatively low dose and following a single injection of cefotaxime), the reduction of the bacterial content was modest (1.7 ± 0.82 log cfu/g, Table II) and transitory (Figure 2). Concerning vancomycin, the reduction of the bacterial content clearly increased with the doses (\( P < 0.001 \); Table II, Figure 3): no bactericidal effect was seen with the lowest dose of vancomycin; the maximal bacterial reduction (5 log cfu/g) was observed with the highest dose of vancomycin.

The times of bacterial regrowth were 6 ± 3 h for cefotaxime. Concerning vancomycin, the time of regrowth was delayed from 1 h to 39 h as the dose increased (\( P < 0.001 \); Table II, Figure 3) The rates of regrowth were similar for cefotaxime and vancomycin regimens.

**Combination therapies**

With the combination, there was no significant additional bacterial reduction when cefotaxime was combined with vancomycin at 1 mg/kg as compared with cefotaxime alone. The combination of cefotaxime plus vancomycin 2.5 and 5 mg/kg led to significantly higher bacterial reduction than each corresponding monotherapy. At a higher dose of vancomycin, the addition of cefotaxime did not lead to higher antibacterial effect than vancomycin alone at the same doses. However, by using the contrasts method, the bacterial reductions obtained with all vancomycin monotherapies were shown to be lower than those observed in the combinations with cefotaxime (\( P < 0.01 \)).
Table II. Pharmacodynamic parameters in infected fibrin clots of cefotaxime or vancomycin and their combinations (means with standard deviation)

<table>
<thead>
<tr>
<th>Regimen*</th>
<th>Time above MIC(a) (h)</th>
<th>Time of regrowth(a) (h)</th>
<th>Concentration at regrowth (mg/g)(a)</th>
<th>(\Delta \log_{10}) cfu at time of regrowth(a)</th>
<th>Rate of regrowth Log cfu/h(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX</td>
<td>9.2 ± 1.4</td>
<td>6 ± 3</td>
<td>0.77 ± 0.47</td>
<td>1.7 ± 0.82</td>
<td>0.25 ± 0.2</td>
</tr>
<tr>
<td>Van 1</td>
<td>17 ± 4</td>
<td>1</td>
<td>0.62 ± 0.65</td>
<td>-0.85 ± 0.24</td>
<td>0.63 ± 0.09</td>
</tr>
<tr>
<td>Van 2.5</td>
<td>18 ± 7</td>
<td>2.3 ± 1.7</td>
<td>1 ± 0.71</td>
<td>0.76 ± 1.2</td>
<td>1.2 ± 1.4</td>
</tr>
<tr>
<td>Van 5</td>
<td>26 ± 6</td>
<td>3.4 ± 1</td>
<td>1.2 ± 0.81</td>
<td>2.75 ± 2</td>
<td>1 ± 1.2</td>
</tr>
<tr>
<td>Van 10</td>
<td>26 ± 5</td>
<td>42 ± 17</td>
<td>0.47 ± 0.56</td>
<td>3.92 ± 0.1</td>
<td>0.03 ± 0.05</td>
</tr>
<tr>
<td>Van 20</td>
<td>28 ± 9</td>
<td>42 ± 12</td>
<td>0.46 ± 0.93</td>
<td>5 ± 0.78</td>
<td>0.03 ± 0.05</td>
</tr>
<tr>
<td>CTX + Van 1</td>
<td>6 ± 1.6</td>
<td></td>
<td>CTX = 0.55 ± 0.25</td>
<td>2.9 ± 2.5</td>
<td>0.85 ± 0.7</td>
</tr>
<tr>
<td>CTX + Van 2.5</td>
<td>10 ± 2</td>
<td></td>
<td>CTX = 0.13 ± 0.1</td>
<td>3.1 ± 2.3</td>
<td>0.54 ± 0.5</td>
</tr>
<tr>
<td>CTX + Van 5</td>
<td>13.5 ± 7.5</td>
<td></td>
<td>CTX = 0.11 ± 0.19</td>
<td>4 ± 0.7</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>CTX + Van 10</td>
<td>36 ± 17</td>
<td></td>
<td>CTX = 0.04 ± 0.05</td>
<td>4.4 ± 1</td>
<td>0.13 ± 0.3</td>
</tr>
<tr>
<td>CTX + Van 20</td>
<td>39 ± 12</td>
<td></td>
<td>CTX = 0.01 ± 0.05</td>
<td>4.9 ± 0.6</td>
<td>0.13 ± 0.11</td>
</tr>
</tbody>
</table>

\(\Delta \log_{10}\) cfu at time of regrowth:
- CTX = 0.55 ± 0.25
- Van = 0.33 ± 0.48
- CTX = 0.13 ± 0.1
- Van = 0.78 ± 0.36
- CTX = 0.11 ± 0.19
- Van = 0.97 ± 0.69
- CTX = 0.04 ± 0.05
- Van = 0.45 ± 0.49
- CTX = 0.01 ± 0.05
- Van = 0.22 ± 0.38

*CTX, Cefotaxime iv 50 mg/kg at hour 0; Van: vancomycin iv at hour 0, numbers denote the dose of vancomycin (mg/kg).

\(\Delta \log_{10}\) Statistical results are presented for each column as follows: first, results of ANOVA; second, if significance was reached \(P < 0.05\), ranking based on the Newman Keuls’s test (the symbol ~ means not different); and third, result of the contrasts' method.

1-ANOVA not significant for vancomycin regimen \((P = 0.25)\).

1-ANOVA: \(P < 0.001\); 2-Van 1 ~ Van 2.5 < CTX = CTX-Van 1 ~ CTX-Van 5 < CTX-Van 10 < CTX-Van 20 = Van 10; 3-contrast not significant for Van-regimens with or without CTX \((P = 0.11)\).

1-ANOVA not significant for Van-regimens \((P = 0.07)\). ANOVA, \(P = 0.001\) for CTX containing regimens; 2-CTX = CTX-Van 1 > all other regimens; 3-contrast significant for CTX-regimens with or without Van \((P < 0.001)\).

1-ANOVA, \(P < 0.001\) for all regimens and also for monotherapies with Van; 2-Van 1 ~ Van 2.5 < CTX = CTX-Van 1 < CTX-Van 2.5 = Van 5 < CTX-Van 5 ~ Van 10 = CTX-Van 20 ~ Van 20; 3-contrast significant for Van-regimens with or without CTX, \(P = 0.01\).

1-ANOVA, \(P = 0.1\).
The times of bacterial regrowth of the combinations increased with the dose of vancomycin. The combination of vancomycin 1 mg/kg with cefotaxime led to the same time of regrowth as that of cefotaxime alone. The addition of cefotaxime to low doses of vancomycin (2.5 and 5 mg/kg) led to a significant delay of the bacterial regrowth compared with vancomycin alone. At higher doses of vancomycin (10 and 20 mg/kg), the times of regrowth with or without cefotaxime were the same. The rates of regrowth were not different whatever regimen was used.

Correlation of pharmacokinetic parameters with therapeutic efficacy

For this analysis, in an attempt to compare all the regimens, the $\log_{10} \text{cfu/g}$ at time of regrowth was used (Table II). The maximal and residual concentrations, the time above MIC, log AUC and the log AUCI were entered into this analysis as independent factors.

For cefotaxime, the factors individually and significantly correlated to efficacy were $C_{\text{max}}$ ($P = 0.02; r^2 = 0.95$), $T > \text{MIC}$ ($P = 0.05; r^2 = 0.88$), AUC ($P = 0.01; r^2 = 0.97$) and AUCI ($P = 0.01; r^2 = 0.97$). For vancomycin, the factors individually and significantly correlated to efficacy were $C_{\text{min}}$ ($P < 0.001; r^2 = 0.49$), AUC ($P = 0.01; r^2 = 0.21$) and AUCI ($P = 0.01; r^2 = 0.21$). The efficacy of the combinations correlated with the $C_{\text{max}}$ of cefotaxime ($P < 0.01; r^2 = 0.4$) and $T > \text{MIC}$ of vancomycin ($P = 0.05; r^2 = 0.2$).

Discussion

Under the experimental conditions used in this study, the level of antibiotic susceptibility of the strain was not altered in the fibrin clots and was the same as that obtained in vitro. Considering that no significant host response exists within the clots (Bergeron et al., 1993), our animal model allowed us to investigate the intrinsic pharmacodynamic parameters of the tested therapeutic regimens on this specific pneumococcal strain. In addition, we chose a constant and relatively low dose of cefotaxime in order to maximize the probability of detecting a higher antibacterial effect when combinations with various doses of vancomycin were tested.

In these in-vivo conditions, as expected, we found that cefotaxime had a modest efficacy at the relatively low concentrations in clots, which were similar to those observed in various human tissues (Shah, Helm & Stille, 1979; Busse, Seeger & Wreesmann, 1980; Danon, 1980; Kafetzis, 1980; Lode et al., 1980; Maesen et al., 1980; Morel, Monrocq & Besnard, 1980; Sarlangue et al., 1984; Lecour et al., 1985). We found also a clear dose-effect relationship of vancomycin on this specific pneumococcal strain. Both these findings are in accordance with our killing curves study in vitro and previous data (Barakett et al., 1992; Barakett, et al., 1993; Cassinat & Nicolas, 1994; Pankuch et al., 1994). More specifically, a multiple linear analysis showed that, in our model, the most important parameter significantly associated with the efficacy of vancomycin was $C_{\text{max}}$ and, both $C_{\text{max}}$ and AUC for cefotaxime.

The main finding of this study was that the combination of cefotaxime plus vancomycin gave a significantly higher antibacterial effect than corresponding monotherapy, if the $C_{\text{max}}$ of vancomycin reached at least 1.9 mg/L. Indeed, if the concentrations of vancomycin were below this level, no increased antibacterial effect was detected as compared with cefotaxime alone. Concentrations of vancomycin
Combined effects of cefotaxime and vancomycin

Figure 3. Bacterial concentrations in fibrin clots (means of at least five experiments in rabbits). ■, Cefotaxime 50 mg/kg at 0 h, alone and ●, with vancomycin. Vancomycin alone (△) iv at 0 h: 1 mg/kg (a), 2.5 mg/kg (b), 5 mg/kg (c), 10 mg/kg (d) or 20 mg/kg (e); ---, control.
between 2 and 2.5 mg/L increased the antibacterial effect significantly of each monotherapy. This improved effect was shown by a greater reduction in the numbers of surviving bacteria and a delay of the time of bacterial regrowth. This latter finding could explain the decrease of the critical concentration at regrowth for cefotaxime in the combinations. With concentrations of vancomycin above 3 mg/L, the addition of cefotaxime did not add any antipneumococcal effect compared with vancomycin alone. All these observations are consistent with previous experimental studies (Barakett et al., 1993; Friedland et al., 1993a; Cassinat & Nicolas, 1994; Friedland et al., 1994; Paris et al., 1994).

Our observations have particular relevance when the diffusion of the drugs is difficult or uncertain. It is especially the case for vancomycin since its tissue diffusion is quite variable (Moellering, Korgstad & Greenblatt, 1981; Schaad, Nelson & McCracken, 1981; Barois et al., 1986; Viladrich et al., 1991; Brinquin et al., 1993) or slow (Barois et al., 1986; Chabenat et al., 1987) even if high doses are given (Barois et al., 1986; Viladrich et al., 1991; Brinquin et al., 1993). In this field further information about dosage, modalities of administration and diffusion of vancomycin are warranted (Klugman, Friedland & Bradley, 1995). Thus, in such clinical conditions, it is conceivable that the therapeutic level of vancomycin could not be reached for the first few hours or even days of the treatment of infection (Barois et al., 1986). During this lag time, the possible consequence is that the conditions of a temporary but ineffective combination therapy may be realized since our results clearly showed that insufficient concentrations of vancomycin gave no additional antipneumococcal effect to cefotaxime alone. So, considering that the prognosis of pneumococcal infections is partly associated with the rate of sterilisation (Geslin et al., 1994a), we suggest that, in case of both a clinical suspicion of highly penicillin-resistant pneumococcal infection and probability of poor antibiotic tissue penetration, vancomycin should be given with a third generation cephalosporin.

Thus, using an in-vivo infection model with a highly penicillin-resistant pneumococcal strain, we demonstrated that the efficacy of vancomycin is related to its maximal concentration and that the combination of cefotaxime and vancomycin gave a higher antibacterial effect than each monotherapy if the maximum concentrations of vancomycin were at least three or four times the MIC.

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References


Combined effects of cefotaxime and vancomycin


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