Comparative study of treatment with penicillin, ceftriaxone, trovafloxacin, quinupristin–dalfopristin and vancomycin in experimental endocarditis due to penicillin- and ceftriaxone-resistant Streptococcus pneumoniae

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The efficacy of different antibiotics was compared in an experimental model of aortic valve endocarditis in rabbits, using a serotype 19 strain of Streptococcus pneumoniae resistant to penicillin (MIC 12 mg/L) and ceftriaxone (MIC 12 mg/L). The results were compared with those of a control group, which received no treatment. One hundred and nineteen animals were treated with one of the following antibiotic regimens: im procaine penicillin G at a dosage of 300 000 U/kg weight/12 h (16 animals); iv trovafloxacin, 13.3 mg/kg/12 h (31 animals); iv ceftriaxone, 75 mg/kg/24 h (21 animals); iv vancomycin, 20 mg/kg/12 h (15 animals) and im quinupristin–dalfopristin, 30 mg/kg/8 h (20 animals). All the antibiotics used in this study proved to be efficient in reducing numbers of S. pneumoniae and in increasing the percentage of aortic vegetations that were rendered sterile compared with the control group. Penicillin at the dosage used in our study was capable of achieving serum concentrations two or three times greater than the MIC, thus demonstrating its effectiveness as an antibiotic for this endocarditis model. No significant difference was observed between the effects of vancomycin, quinupristin–dalfopristin and penicillin. Vancomycin proved to be more efficient than trovofloxacin in reducing the bacterial load and increasing the numbers sterilized. There was also a tendency for this antibiotic to be more effective than ceftriaxone in reducing the bacterial load of the vegetations. There was a statistically significant correlation between the weight of the vegetations and their bacterial load. In the light of these results, vancomycin and quinupristin–dalfopristin may be considered suitable alternatives to penicillin for the treatment of penicillin-resistant S. pneumoniae endocarditis.

Introduction

Streptococcus pneumoniae is an infrequent cause of bacterial endocarditis, being responsible for <3% of adult cases.1–3 Recent studies reveal an alarming increase in the number of penicillin-resistant pneumococcal infections worldwide and cases of endocarditis caused by these bacteria have already been described.4–6 In the USA, for example, the levels of resistance are c. 44% and in France 42.8%.7,8 Spain, too, has one of the highest resistance rates. According to the study published by the pneumococcal reference laboratory of the Centro Nacional de Microbiología in Majadahonda (Spain),9 49% of the strains studied revealed a diminished susceptibility to penicillin (MIC > 0.06 mg/L), and most of these were also resistant to erythromycin, tetracyclines, chloramphenicol and third-generation cephalosporins.5,10–12

Parenteral administration of penicillin has long been the treatment of choice for serious pneumococcal infections, but the existence of cases of endocarditis caused by penicillin-resistant strains of S. pneumoniae presents a new challenge. Other traditional antibiotics such as vanco-
mycin, ceftriaxone and imipenem have been proposed for treatment. Others, such as trovafloxacin or quinupristin–dalfopristin, have recently appeared and may constitute valid alternatives for treating these infections.

In previous studies using an experimental endocarditis model, the efficacy of cefotaxime, teicoplanin and penicillin at different doses, on two strains of \textit{S. pneumoniae} with differing sensitivity to penicillin (MIC 1 and 4 mg/L) were studied. Our objective in the present study was to compare the therapeutic efficacy of penicillin, vancomycin, ceftriaxone, trovafloxacin and quinupristin–dalfopristin against a strain of \textit{S. pneumoniae} with high-level resistance to penicillin and ceftriaxone (MIC 12 mg/L for both) in an experimental model of aortic valve endocarditis in rabbits.

### Materials and methods

#### Microorganism

The strain of \textit{S. pneumoniae} used had originally been isolated by bronchoalveolar lavage of a male, HIV-infected patient who was admitted to hospital with a respiratory tract infection. The isolate belonged to serogroup 19.

#### In vitro studies

Antimicrobial susceptibility tests were performed using three different methods to determine the MIC: (i) microdilution in broth following the NCCLS criteria, (ii) an Etest (AB Biodisks, Solna, Sweden) according to the manufacturer’s instructions and (iii) dilution in Mueller–Hinton agar, supplemented with 5% lysed horse blood, incubated at 35°C in an aerobic atmosphere. \textit{S. pneumoniae} strain ATCC 49619 was used as the control strain.

#### Experimental endocarditis

All the animal studies were carried out in accordance with the Scientific Procedures Act (Animals) 1986 and the Codes of Practice for the Housing and Care of Animals Used in Scientific Procedures, 1989.

Endocarditis was induced in 145 New Zealand rabbits each weighing 2 kg, using Garrison & Freedman’s technique as modified by Durack & Beeson. The animals were anaesthetized with 20 mg/kg of ketamine (Ketolar, Parke-Davis) through one of the marginal veins of the ear. Then, a right paratracheal cervicotomy was performed involving dissection and exteriorization of the right common carotid artery. A sterilepolyethylene catheter (Cencath Vigon BP, 795440 Ecouen, France) with an internal diameter of 0.8 mm and an external diameter of 1.2 mm was introduced into the left ventricle, where it remained throughout the experiment. The catheter was considered to be correctly placed when it transmitted the heartbeat in a constant manner.

Twenty-four hours after insertion, 1 mL of saline solution containing an inoculum of 10⁸ cfu \textit{S. pneumoniae} (resistant to penicillin) was injected into the marginal ear vein.

#### Blood cultures

Immediately before antibiotic treatment was started, blood was obtained for culture by inoculating 10 mL tryptic soy broth with 0.4 mL of blood withdrawn through an ear vein. After 24 h, a preliminary macroscopic examination was carried out. The blood cultures were considered positive if the characteristic colonies were seen to be growing on the plates. Those rabbits in which no positive blood cultures were obtained were excluded from the study.

#### Antimicrobial treatment

The animals were divided into five study groups: 16 in the control group, 31 in the trovafloxacin group, 15 in the vancomycin group, 16 in the penicillin group, 21 in the ceftriaxone group and 20 in the quinupristin–dalfopristin group. Antibiotic treatment was initiated 24 h after administration of the inoculum and immediately after a blood sample had been taken for culture. The animals were treated for 5 days with the following doses of antibiotics and methods of administration: iv trovafloxacin (Trovan, Pfizer Laboratories, Madrid, Spain) 13.3 mg/kg every 12 h; iv vancomycin (Diatracin, Distalaboratories, Madrid, Spain) 20 mg/kg every 12 h; iv ceftriaxone (Rocefalin, Roche Laboratories, Barcelona, Spain) 75 mg/kg every 24 h; im penicillin G procaine (Farmaproina, Cepa Laboratories, Madrid, Spain) 300 000 U/kg every 8 h and im quinupristin–dalfopristin (Synercid, Rhône Poulenc Rorer Laboratories, Collegeville, PA, USA) at a dosage of 30 mg/kg every 8 h. The rabbits in the control group received no antibiotic treatment.

#### Examination of vegetations

Eight hours after the last dose of the 5 day drug regimen, each animal was killed by rapid injection of 150 mg thiopental (Pentothal Sódico, Abbott Laboratories, Chicago, IL, USA) through a marginal vein in the ear. Only those animals that survived at least 24 h after bacterial challenge were included in the study. The ribcage was immediately opened and the heart was removed, the correct position of the catheter being checked at the same time. A macroscopic diagnosis of endocarditis was performed by observing the characteristic vegetations. Only those animals that survived at least 48 h after introduction of the catheter were included in the study. Those rabbits with incorrectly placed catheters or showing no vegetations were excluded.

Under aseptic conditions, the vegetations were resected from the aortic leaflets, weighed and homogenized in 0.9 mL sterile physiological saline. A series of 10-fold
Treatment of experimental *S. pneumoniae* endocarditis
dilutions was then prepared in saline and 100 μL of each
dilution was plated on to blood agar. Colonies were
counted after 48 h incubation at 37°C in air and expressed
as log_{10} cfu/g. Cultures were considered sterile if there were
<2 log_{10} cfu/g. The limit of detection of the method used
was 100 cfu/g and so sterile cultures were assigned a log_{10}
value of 2. The microorganisms were identified by the
characteristic appearance of the colonies.

Response to treatment
For inclusion in the analysis, blood cultures had to show
the presence of *S. pneumoniae* before treatment, and each
animal had to survive 5 days and show vegetations. The
response to therapy was determined by the overall survival
rate, the sterilization of blood cultures and vegetations and
the number of cfu/g *S. pneumoniae* in each vegetation.

Twenty-six animals were excluded from further con-
sideration in the study for the following reasons: three
were from the control group (because of negative blood
cultures); eight from the trovafloxacin group (five because
of negative blood cultures and three deaths before 48 h);
two from the vancomycin group (one because of negative
blood cultures and one not showing vegetations); six from
the ceftriaxone group (three negative blood cultures, one
death before 48 h and two without vegetations); and six
from the quinupristin–dalfopristin group (two negative
blood cultures and four deaths before 48 h).

The cause of death was related directly to surgical inter-
vention in five cases (three cardiac cavity perforations and
two profuse bleeding from neck blood vessels) and un-
known in the other four cases. Once the first 48 h had
passed (24 h after infection) no mortality occurred in any of
the groups, so that 119 rabbits remained for analysis.

Antibiotic serum levels
After 5 days of treatment, blood samples were taken from
all the rabbits through the marginal vein in the ear. The
samples were taken on two occasions: (i) 1 h after the
administration of the antibiotic when it was given iv, or
1.5 h after im administration (peak value); and (ii) im-
mediately before the administration of the next dose of the
antibiotic (trough value). The drug concentrations in plasma
were measured by a bioassay method using *Staphylococcus
aureus* ATCC 25932 for penicillin, *Micrococcus luteus*
ATCC 9341 for quinupristin–dalfopristin, *Escherichia coli*
ATCC 25992 for ceftriaxone and *Bacillus subtilis* ATCC
6633 for trovafloxacin.\(^27,28\) Competitive enzymo-immuno-
analysis with monoclonal antibodies was used to determine
the concentrations of vancomycin in an ACA IV discrete
clinical analyser (Dabe Behring).

Statistical analysis
The χ² test was used to determine the relationship between
qualitative variables, or Fisher’s exact test when the ex-
pected results were under five. Bonferroni’s correction was
applied when multiple comparisons were performed. For
the comparison of averages between each group of treat-
ment and the control group the Mann–Whitney U-test was
used. The overall comparison of averages between the
study groups was determined by means of the Kruskal–
Wallis test, with subsequent contrast analysis using
Dunnett’s test. The correlation between two qualitative
variables was calculated using Pearson’s \(r\) coefficient. Sta-
tistical significance was considered to exist when \(P < 0.05\),
except in correlation analysis, when a \(P < 0.01\) was
required.

Results
In vitro studies
The MICs for the test strain of *S. pneumoniae* were as
follows: penicillin 12 mg/L, cefotaxime 6 mg/L, ceftriaxone
12 mg/L, erythromycin 4 mg/L, vancomycin 0.5 mg/L, trova-
floxacin 0.19 mg/L and quinupristin–dalfopristin 0.75 mg/L.

Table I shows the MICs of the different antibiotics for
*S. pneumoniae* and the average peak and trough serum
levels of each antibiotic. The values of quinupristin–
dalfopristin and the trough values of trovafloxacin were
<4 and 0.5 mg/dL, respectively, these being the minimum
values detectable by the technique used.

<table>
<thead>
<tr>
<th>Group</th>
<th>MIC (mg/L)</th>
<th>Peak serum concentration (mg/L)(^a)</th>
<th>Trough serum concentration mg/L(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trovafloxacin</td>
<td>0.19</td>
<td>4.21 ± 1.33</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.5</td>
<td>38.60 ± 4.20</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>12</td>
<td>294 ± 27</td>
<td>8.1 ± 2.2</td>
</tr>
<tr>
<td>Penicillin</td>
<td>12</td>
<td>27 ± 4.3</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td>Quinupristin–dalfopristin</td>
<td>0.75</td>
<td>&lt;4</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± s.d.
Table II. Results of treatment of experimental endocarditis due to penicillin- and ceftriaxone-resistant *S. pneumoniae* (MIC 12 mg/L for both)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Weight of vegetations mg&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt; cfu/g vegetation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sterile vegetations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>200.63 ± 235</td>
<td>8.69 ± 0.53</td>
<td>0</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>31</td>
<td>80.45 ± 53.35</td>
<td>3.98 ± 2.37</td>
<td>51.6</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>15</td>
<td>38.60 ± 25.75</td>
<td>2.00 ± 0.00</td>
<td>100</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>21</td>
<td>64.62 ± 36.92</td>
<td>3.56 ± 2.24</td>
<td>61.9</td>
</tr>
<tr>
<td>Penicillin</td>
<td>16</td>
<td>112.94 ± 85.79</td>
<td>2.79 ± 1.82</td>
<td>81.2</td>
</tr>
<tr>
<td>Quinupristin–dalfopristin</td>
<td>20</td>
<td>95.90 ± 55.24</td>
<td>2.54 ± 1.37</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± s.d.

**Experimental endocarditis**

Table II shows the weight of the vegetations, the log<sub>10</sub> of the cfu/g of vegetation and the percentage of sterile vegetations in all the groups.

The average weight of the aortic vegetations was 95.5 mg. The highest average of all the groups was observed in the control group, while the lowest was seen in the vancomycin group followed by the ceftriaxone and trovafloxacin groups. No significant differences were found between the weight of the vegetations of the control group and the penicillin or quinupristin–dalfopristin groups, while such differences were significant between the control group and the other three treatment groups (trovafloxacin, ceftriaxone and vancomycin).

*S. pneumoniae* were observed in the vegetations of all the rabbits of the control group, meaning that there was no spontaneous healing. The numbers sterilized in all the groups attained statistical significance in comparison with the control group (*P* < 0.001). Between the groups themselves there were only significant differences between the vancomycin and trovafloxacin group (*P* < 0.05).

All the antimicrobials were to a greater or lesser extent effective in significantly reducing the bacterial load of the vegetations. The average of the log<sub>10</sub> cfu/g of vegetations in the control group was the highest of all the groups, followed by those observed in the trovafloxacin, ceftriaxone, penicillin, quinupristin–dalfopristin and vancomycin groups. The average cfu/g in the trovafloxacin group was only significantly different from the average values observed in the control and vancomycin groups. The vancomycin group revealed the greatest decrease in average cfu/g (6.69 points below the value of the control group), although the differences were only significant between it and the control and trovafloxacin groups; the differences were on the limit of significance with respect to the ceftriaxone group. The differences observed in the penicillin and quinupristin–dalfopristin groups were only significant when compared with the control group.

A comparative analysis between the penicillin and control groups revealed no significant differences between the weight of vegetations in the respective groups, although there were significant differences (*P* < 0.001) with regard to the bacterial load and numbers of vegetations sterilized.

A similar comparative analysis between the quinupristin–dalfopristin and control groups revealed no significant differences between the weight of the vegetations, although, once again, the differences were significant (*P* < 0.001) when the bacterial load and numbers sterilized (*P* < 0.001) were compared.

When the trovafloxacin and the control groups were compared, significant differences were seen to exist between the respective weights of the vegetations (*P* < 0.05), the bacterial load (*P* < 0.001) and numbers sterilized (*P* < 0.001).

The average weight of the sterile vegetations was 77.91 ± 61.6 mg, and that of their non-sterile counterparts 124.4 ± 154 mg; statistical analysis showed that significant differences existed between both groups (*P* < 0.05), and when a comparative analysis between the weight of the vegetations and their bacterial load was performed, the result was a correlation of 0.295 at *P* < 0.001. Thus, it may be stated that there is a significant correlation between the weight of the vegetations and their bacterial load.

**Discussion**

All the antibiotics used in this study proved to be effective in decreasing the concentration of *S. pneumoniae* and in increasing the numbers of aortic vegetations rendered sterile in comparison with the control group. In this respect there were no significant differences between vancomycin, quinupristin–dalfopristin and penicillin. Vancomycin was more effective than trovafloxacin in reducing the bacterial load and in increasing the numbers sterilized; it also tended to be more effective than ceftriaxone in reducing the bacterial load of the vegetations. There was significant correlation between the weight of the vegetations and their bacterial load. The results of our study revealed that vanco-
mycin and quinupristin–dalfopristin are effective antimicrobials in the treatment of this model of endocarditis, and so may be considered valid alternatives to penicillin for treating the type of endocarditis studied.

To date, the only study of an experimental model of penicillin-resistant *S. pneumoniae* is that published by Fernández Guerrero *et al.*23 These authors used two different strains of pneumococci with different sensitivities to penicillin (MIC 1 and 4 mg/L), and studied the effectiveness of penicillin, cepotaxime and teicoplanin. Their observations suggest that the animals treated with doses of penicillin G procaine that achieved serum levels near the MIC for pneumococci, showed a significant reduction in log<sub>10</sub> cfu/g of vegetation compared with the control group, although only 20% of the animals showed sterile vegetations. When the serum levels of penicillin were three to four times the MIC, there was a greater reduction in log<sub>10</sub> cfu/g of vegetation, and 88% of the animals showed sterile vegetations. Only the doses of penicillin that maintained serum concentrations above the MIC between doses provided constant sterilization of the cardiac vegetations. In terms of antimicrobial efficacy, cepotaxime and teicoplanin induced a similar response to that produced by high doses of penicillin. There are no studies in the literature comparing vancomycin, ceftriaxone and quinupristin–dalfopristin with penicillin. It is interesting to note that penicillin administered at the doses used in our study is able to produce serum concentrations two or three times greater than the MIC and represents an effective antimicrobial for the treatment of this type of endocarditis. It was placed third as regards its capacity to decrease the cfu/g of vegetation and sterilized 81.2% of the vegetations. The serum concentrations obtained with this antibiotic were higher than those obtained by Fernández Guerrero *et al.*23 using the same dose. These differences may be explained partly by the fact that the above authors took their blood samples to measure penicillin levels 1 h after the im administration of the drug, whereas we took them 1.5 h afterwards. Penicillin trough levels were below the MIC, which would explain why fewer than 100% of the vegetations were sterilized, since only doses that can provide serum concentrations above the MIC on a continuous basis can have a continuous sterilization effect.25 It is known that patients with pneumococcal meningitis caused by strains highly resistant to penicillin do not respond to treatment with penicillin or ampicillin because neither reaches sufficiently high concentrations in the cerebrospinal fluid to eliminate these resistant strains.29,30 However, penicillin can reach high concentrations in the region of cardiac vegetations, which explains why it may still be effective in the treatment of endocarditis.

Quinupristin–dalfopristin was shown to be an effective antibiotic in this model of endocarditis, with no significant differences being seen between it and vancomycin and penicillin. After vancomycin, it was the most effective antimicrobial in reducing the cfu/g of vegetation, sterilizing 85% of the aortic vegetations. This streptogramin has the advantage of not being affected by the resistance of the pneumococci to penicillin and it has demonstrated its value as an alternative antibiotic in the treatment of infections caused by penicillin-resistant *S. pneumoniae*.19,20,31 The method used to measure the serum concentrations allowed us to determine only that they were less than 4 mg/L. In previous experimental endocarditis studies in the rabbit,21 using the same dose per kg and the same time intervals and methods of administration, peak values of 3.0 ± 0.8 mg/L were obtained, which are similar to our findings, and three to four times above the MIC. Quinupristin–dalfopristin has consistently demonstrated a postantibiotic effect against Gram-positive bacteria, and the duration of this effect appears to be dependent on drug concentration rather than on the length of exposure.32,33 This effect should be borne in mind when evaluating the results, to ascertain the drug’s effect on the decrease in the log<sub>10</sub> cfu/g of vegetation.

Trovafloxacin was chosen for this study because it is the most active of all the new fluoroquinolones against *S. pneumoniae* and because it shows no cross-resistance with penicillin.16,34,35 It has already produced good results in the case of *S. pneumoniae* penicillin-resistant pneumonia and meningitis.17,18 In our study, serum peak levels were between 14 and 32 times higher than the MIC and trough levels were similar. Trovafloxacin was shown to be effective in reducing the bacterial load and in rendering vegetations sterile (51.6%), although it was significantly less effective than vancomycin in reducing both parameters. This study was made before the use of the drug in Europe was provisionally suspended due to the existence of important side effects and the death of some patients that may have been related to its use.

Ceftriaxone was included in the study because of the increased resistance of *S. pneumoniae* to cephalosporins and because it is frequently recommended for this type of infection.3,13 High serum concentrations can be obtained with this drug.15 The peak serum concentrations are 22–25 times above the MIC, and the trough values slightly above the MIC. In a comparison between ceftriaxone and vancomycin, there was a tendency for the latter to be more effective than the former only in reducing the bacterial load. Ceftriaxone was able to sterilize 61.9% of the vegetations and was more effective than trovafloxacin only in reducing the cfu/g of vegetation. Given that ceftriaxone is very highly bound to serum proteins, high concentrations in serum would not reflect high concentrations of free drug. In addition, the concentrations of this antibiotic found in vegetations vary between 18 and 47% of serum concentrations. Taken together these observations might partly explain the lesser effect of ceftriaxone with respect to penicillin, observed in the present study.26

Vancomycin sterilized all the animals, which confirms its usefulness for the control of infections caused by resistant *S. pneumoniae*.13–15 as this microorganism continues to be very susceptible to vancomycin, with an MIC of <0.5 mg/L.
Serum concentrations remained above the MIC throughout the 24 h, with peak values 68–84 times higher than the MIC. Comparative analysis with penicillin and quinupristin–dalfopristin did not reach statistical significance, and we were unable to conclude whether one drug was more effective than the other.

There was a close relationship between the weight of the vegetations and their bacterial load. Thus, a correlation exists between their size and the therapeutic effectiveness of agents used, as judged by the cfu/g and the rate of sterilization of the vegetations.

Subsequent research should evaluate the combined use of the different antibiotics used in this study with, for example, aminoglycosides.

Although we used the infectious endocarditis model in rabbits due to its simplicity, reproducibility, relatively low cost, the possibility of using large numbers of animals and because of its histological similarity to human endocarditis, there are differences between human endocarditis and that produced experimentally in rabbits, so great care should be taken before extrapolating these results to humans.

References

Treatment of experimental *S. pneumoniae* endocarditis


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