In vitro susceptibility of vancomycin-resistant enterococci (VRE) to fosfomycin

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Received 28 August 2001; accepted 10 January 2002

Abstract

We evaluated the in vitro activity of fosfomycin against 75 clinical isolates of vancomycin-resistant enterococci (VRE). Using the NCCLS breakpoint for susceptibility of urinary tract isolates to fosfomycin (MIC $\leq$ 256), 51 out of 52 \textit{Enterococcus faecium} and all \textit{Enterococcus faecalis} isolates tested were susceptible or intermediate to fosfomycin. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Fosfomycin is a phosphonic acid bactericidal agent that acts primarily by inhibiting bacterial cell wall peptidoglycan synthesis (Patel et al., 1997). The compound is active against many urinary pathogens including \textit{Escherichia coli}, \textit{Staphylococcus saprophyticus} and many strains of enterococci (Hamilton-Miller, 1991; Med. Let. Drugs Ther., 1997; Patel, 1997). Published MIC\textsubscript{90} of fosfomycin against enterococci are 52.3 $\mu$g/ml (Patel et al., 1997). There is little information on the activity of fosfomycin vs. VRE. In recent years, enterococci have emerged as significant clinical pathogens with both intrinsic and acquired resistance to a number of antibiotics. In view of the limited treatment options for VRE, in this study we evaluated the in vitro susceptibilities of fosfomycin versus 75 clinical isolates of VRE.

2. Materials and methods

We tested 75 clinical isolates of VRE. The strains of VRE used in this study were clinical isolates obtained from hospitalized patients from 1991 to 1998 from different U.S. hospitals. All isolates were resistant to multiple other agents including ampicillin and high-levels of gentamicin. Fifty-two isolates were \textit{E. faecium} and 23 isolates were \textit{E. faecalis}. Twenty-two isolates were from urine, 18 isolates were from unknown/other sources, 16 isolates were from blood, 12 isolates were from wounds/abscesses, 6 isolates were from peritoneal fluid and one isolate was from nasal secretions.

The bacteria were identified as enterococci using conventional biochemical tests as described by Facklam and Collins (Facklam & Collins, 1989). All isolates were stored in BHI/glycerol broth at $\pm$70°C. Fosfomycin (Forest Pharmaceuticals, Inc., St. Louis, MO) was obtained in the form of standard laboratory powder and was stored at $\pm$70°C before use. Fosfomycin E-strips (lot no.B92692) were purchased from AB Biodisk North America Inc., Piscataway, NJ. Fosfomycin E-strips contain glucose-6-phosphate (25 $\mu$g/ml).

MICs for fosfomycin were determined for each isolate by E-test (Fuchs et al., 1999) and a previously described microtiter broth dilution method (Barry & Fuchs, 1991) and according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS, 1999). The organisms were incubated for 20h in Mueller-Hinton broth and appropriate dilutions were made to obtain a final inoculum of $5 \times 10^5$ CFU/mL. Antibiotics were prepared manually in cation-supplemented Mueller-Hinton broth supplemented with 25 $\mu$g/ml of glucose-6-phosphate and...
were dispensed automatically with an MIC-2000 dispenser (Dymatech Laboratories, Inc., Alexandria, VA). Concentrations of fosfomycin tested ranged from 64 \( \mu \)g/ml to 1024 \( \mu \)g/ml. The MIC was described at the lowest concentration of drug that prevented visible growth after 18h of incubation. Interpretations of in vitro resistance were made according to NCCLS. The cutoffs for resistance to fosfomycin for treatment of urinary tract infection are \( \geq 256 \) \( \mu \)g/ml and sensitive is defined as MIC \( \leq 64 \) \( \mu \)g/ml.

### 3. Results

The MICs for the 52 E. faecium isolates ranged from 8 to 256 \( \mu \)g/ml. The MICs for the 23 E. faecalis isolates ranged from 16 to 64 \( \mu \)g/ml (broth microdilution) (see Table 1). Results of E-strip tests were more consistent with broth microdilution for E. faecalis isolates (MIC range 12–64 \( \mu \)g/ml). Although the overall susceptibility rate (67%) was similar among the E. faecium isolates some variation was seen between the broth microdilution and E-test results. None of the E. faecium isolates were resistant by E-test (Table 2).

Vancomycin genotype was known for 34 isolates (18 were vanA and 16 were vanB). For vanA isolates, fosfomycin MICs ranged from 8 to 128 \( \mu \)g/ml. One isolate had MICs = 8 \( \mu \)g/ml, 3 isolates had MICs = 32 \( \mu \)g/ml, 10 isolates had MICs = 64 \( \mu \)g/ml and 4 isolates had MICs = 128 \( \mu \)g/ml. For vanB isolates, fosfomycin MICs ranged from 16 to 128 \( \mu \)g/ml. One isolate had MICs = 16 \( \mu \)g/ml, 5 isolates had MICs = 32 \( \mu \)g/ml, 7 isolates had MICs = 64 \( \mu \)g/ml and 3 isolates had MICs = 128 \( \mu \)g/ml.

### Table 2

<table>
<thead>
<tr>
<th>No. of strains</th>
<th>E-test MICs (( \mu )g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>E. faecium</td>
<td>52</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
</tr>
</tbody>
</table>

### 4. Discussion

Fosfomycin has been available in Europe for many years as a parenteral compound, fosfomycin disodium. Two other compounds are available for oral administration: fosfomycin calcium and fosfomycin troetamol. Fosfomycin troetamol is the result of systematic studies aimed at improving the biopharmaceutical properties and, among all the phosphonic acid derivatives, is the compound with the most favorable bioavailability profile.

Fosfomycin troetamol has the attributes considered desirable in an agent to be used for the single-dose therapy of uncomplicated lower UTI; these include a broad spectrum of activity against common urinary pathogens, high and prolonged urinary concentrations, retention of antibacterial activity in the presence of urine, rapid bactericidal activity, little or no tendency to induce or select for resistant strains, and good tolerability (Bergan et al., 1990). There is limited information on treatment of VRE, however, a successfully treated complicated VRE urinary tract infection treated with fosfomycin has been reported (Shrestha et al., 2000).

In multiple dose use studies, resistance to fosfomycin emerges rapidly but cross-resistance with other antimicrobials has been uncommon, possibly due to its unique chemical structure and mechanism of action (Suarez et al., 1991; Greenwood et al., 1990). Bacterial resistance to fosfomycin can be either chromosomal or, more rarely, plasmid mediated. Fosfomycin is taken into cells by active transport through the partially constitutive L-2-glycerophosphate uptake system and by a secondary transport system which mediates hexose monophosphate uptake. Most chromosomally resistant mutants have an impairment in one or both of these uptake systems (Suarez et al., 1991). The use of fosfomycin has selected another type of resistant organisms which actively incorporate the drug and have a fully sensitive target enzyme indicating that their mechanism of resistance is different from that of the previously described mutants. They are usually multiresistant, can transfer their resistance through conjugation or transformation, and can be made susceptible through the use of curing agents, indicating a plasmid location for the fosfomycin resistance determinant.

All VRE faecalis tested in this study were susceptible to fosfomycin and 35 out of 52 (67%) VRE faecium isolates in our study were susceptible to fosfomycin and 16 (31%) were intermediate (MIC = 128 \( \mu \)g/ml) as determined by broth microdilution. No apparent association was noted between presence of vanA or vanB genes and fosfomycin susceptibility. Majority of the isolates were from urinary tract and source of the isolates was not associated with higher MICs to fosfomycin. Isolates of VRE we studied were from both before and after FDA approval of fosfomycin in the United States. Fosfomycin troetamol has only been approved for use for treating single, uncomplicated episodes of lower urinary tract infections. Our study has only addressed the issue of in vitro susceptibilities of VRE.
to fosfomycin. Given the high rate of VRE susceptibility to fosfomycin and the good tolerability and high urinary concentrations of the drug, fosfomycin may be considered for treatment of uncomplicated VRE lower urinary tract infections. Further studies will be needed to address the clinical usefulness of fosfomycin.

References


