In vitro activity of fosfomycin in combination with vancomycin or teicoplanin against *Staphylococcus aureus* isolated from device-associated infections unresponsive to glycopeptide therapy

Attività in vitro di fosfomicina in associazione con vancomicina o teicoplanina nei confronti di Staphylococcus aureus *isolati da infezioni associate a device non responsive a terapia con glicopeptidi*

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■ **INTRODUCTION**

Staphylococcus aureus produces a variety of syndromes with clinical manifestations ranging from pustule formation to sepsis and death. Device-associated (central venous catheter, vascular graft, prosthetic cardiac valves, ventricular shunts and orthopaedic prostheses) infections (DAI) are a major concern worldwide. DAI often persist until the device is removed (even if the given antibiotic is active against the causative micro-organism), and the preferential bacterial colonization of biomaterials and local immunodeficiency explain the high infective susceptibility of implants and persistence of infection [1-4]. Bacterial species develop a survival mode by adhering to inert surfaces and producing biofilm; *Staphylococcus aureus* is a very frequent pathogen of such infections and methicillin-resistance is encountered with increasing frequency [5, 6]. In recent years, as a possible consequence of wider glycopeptide usage, minimum inhibitory concentrations (MICs) increasing for vancomycin and teicoplanin have been documented [7, 8].

Glycopeptides remain the agents of choice for infection as severe as methicillin-resistant *Staphylococcus aureus* (MRSA) DAI but the persistence of *S. aureus* bacteraemia under vancomycin alone or in combination with other compounds, despite therapeutic serum concentrations, and the phenomenon of increasing MICs for glycopeptides, which may be a step towards the emergence of resistance, has prompted many authors to study alternative agents to vancomycin or new antibiotic combinations [8-11].

Fosfomycin is a phosphoenolpyruvate analogue produced by *Streptomyces* that irreversibly inhibits enolpyruvate transferase, which prevents the formation of N-acetylmuramic acid, an essential element of the peptidoglycan cell wall [12]. This antibacterial process is dissimilar from the action of beta-lactam antibiotics and glycopeptides. It shows broad-spectrum bactericidal activity that includes *Staphylococcus* spp. [13].

We undertook the present study to evaluate the *in vitro* bactericidal activity of fosfomycin alone and in combination with teicoplanin or vancomycin at different multiples of MIC against MRSA strains isolated from patients with DAI unresponsive to glycopeptide therapy.

■ MATERIALS AND METHODS

The organisms studied were MRSA clinical isolates from patients with DAI unresponsive to glycopeptide therapy despite device removal who were hospitalized at the "Policlinico Umberto I" University Hospital in Rome [14].

One strain was isolated from a case of meningitis secondary to indwelling lumbar drainage who relapsed after two courses (two weeks and two weeks, respectively) with full vancomycin dosages despite prompt removal of the infected drain. Four strains were isolated from one epicardial pacing wire and three orthopaedic prosthesis infections relapsed despite device removal and subsequent 6-8 weeks of glycopeptide therapy. One strain was isolated from post-cardiosurgery mediastinitis originating from a contaminated epicardial pacing wire: the patient died due to persisting bacteraemia under appropriate vancomycin therapy despite surgical debridment and device removal. In the remaining case, removal of the infected device was considered hazardous by the surgeons: the strain was isolated from a patient with ascending aorta vascular graft infection and bacteraemia persisting under vancomycin therapy. After the addition of co-trimoxazole bacteraemia cleared, but the patient eventually died.

Susceptibility to oxacillin and other antibiotics was tested by both the agar diffusion (Kirby-Bauer) and microdilution methods, according to NCCLS criteria [15]. The sensitivity of the strains against vancomycin, teicoplanin, and fosfomycin was assessed by duplicate measurements of MICs by macrobroth dilution following previously described methods [16]. A MIC ≥128 mg/L characterizes a strain as resistant, 32-64 mg/L as intermediate and ≤16 mg/L as susceptible [13]. Drugs were freshly prepared for each experiment. All experiments were performed in cation-supplemented Mueller-Hinton (CSMHB) (Difco Lab, USA). Time-killing studies were carried out in duplicate by modified methods previously described [16]. A colony of bacteria from an overnight plate was suspended and let grow in CSMHB until the McFarland turbidity of 1 was reached; the solution was diluted and starting inoculum 6.12 (SD 0.26) (Log_{10} cfu/mL) for the seven MRSA strains. Flasks were incubated at 35°C, viable counts were determined at 0, 6, and 24 h. Because all strains have been isolated from the same institution we also performed Sma1 endonuclease digested DNA fragments separation by pulsed-field gel electrophoresis (PFGE) with a CHEF-DR II apparatus (Bio-Rad, Hercules, California, USA) to study clonality of the strains. We used the methods previously described [17].

■ RESULTS

Table 1 shows the MICs for vancomycin, teicoplanin and fosfomycin. All strains were inhibited by relatively high MICs for vancomycin (range 1-4 µg/mL) and teicoplanin (range 2-8 µg/mL). Only two strains (CRL, RZZ) were

Table 1 - Minimal MICs (ìg/mL) of the agents studied against MRSA from infection unresponsive or slowly responsive to glycopeptide therapy.

Strain	Device	Teicoplanin	Vancomycin	Fosfomycin
SCR	Epicardial pacing wire	4	2	≥64
HMT	Lumbar drainage	4	4	≥64
CRL	Lumbar drainage	4	4	≥64
ANG	Hip prosthesis	8	2	32
TVL	Epicardial pacing wire	8	4	64
GMB	Knee prosthesis	2	2	≥64
RZZ	Hip prosthesis	8	1	8

Table 2 - Bactericidal activity of teicoplanin, vancomycin and fosfomycin alone or in combination at multiples of MIC against MRSA strains.

Drug concentration (multiples of MIC)	Inoculum	Viable count (Log$_{10}$ cfu/mL)	
		After 6 h	After 24 h
Teicoplanin (2xMIC)	6.12 ± 0.26	6.30 ± 1.00	6.32 ± 0.91
Vancomycin (2xMIC)	6.12 ± 0.26	6.20 ± 1.20	6.36 ± 0.46
Fosfomycin (MIC)	6.12 ± 0.26	5.99 ± 1.59	6.90 ± 1.92
Vancomycin (1xMIC) + fosfomycin (8 μg/mL)	6.12 ± 0.26	5.95 ± 1.07	3.90 ± 0.78
Teicoplanin (1xMIC) + fosfomycin (8 μg/mL)	6.12 ± 0.26	5.00 ± 0.45	4.00 ± 0.79

susceptible to fosfomycin, two strains had an intermediate susceptibility (ANG, TVL) and three were resistant [18] (SCR, HMT, GMB).

Table 2 summarizes the results obtained from the time-kill study. No bactericidal activity of the glycopeptide antibiotics was seen when used alone and higher concentrations did not result in an augmented bactericidal effect. Analogously, fosfomycin was not bactericidal at baseline concentrations (MIC). The combination of a glycopeptide plus fosfomycin demonstrated bactericidal activity already at a concentration of glycopeptide 1 x MIC + fosfomycin 8 μg/mL. At 24 h the combination resulted in more than 2 log$_{10}$ reduction in viable bacteria.

Molecular genotyping by PFGE of isolates revealed that none of the banding patterns were identical. Four strains showed completely different PFGE patterns. However, three (SCR, HMT and TVL strains) isolates showed a similar pattern, differing in two to five bands from the previously described Iberian clone [17]. It must be remarked that these three strains were all resistant or intermediate to fosfomycin. Our data confirm the hypothesis of worldwide clonality of MRSA strains [11, 17].

■ DISCUSSION

Vancomycin and teicoplanin still represent the drugs of choice for treatment of severe infection caused by MRSA. However, some reports indicate that glycopeptide are probably a less efficacious therapy for MRSA than penicillinase-stable penicillins are for methicillin-susceptible *Staphylococcus aureus* [19]. Furthermore, MRSA strains with reduced susceptibil-

ity to vancomycin associated with treatment failure of vancomycin have been isolated in Japan and the U.S. [8, 11, 20]. In particular, a recent analysis of 42 cases of MRSA septicaemia showed poor *in vivo* activity of vancomycin [21].

All MRSA strains tested in the present study were inhibited by relatively high MICs for vancomycin and teicoplanin. We would stress that MRSA with such reduced susceptibility to glycopeptide were not observed among our clinical isolates from 1986 to 1992 [7]. On the other hand during 1993-94, out of 91 MRSA as many as 34 strains were inhibited by MICs of 2 to 4 mg/mL for teicoplanin and 2 strains by a MIC of 2 mg/mL for vancomycin [7]. None of those strains reported MICs as high as 8 mg/mL for teicoplanin or 4 mg/mL for vancomycin as did some of the MRSA strains tested in the present series. No bactericidal activity of glycopeptide antibiotics was seen when used alone against the clinical isolates, and higher concentrations of glycopeptide did not result in increased bactericidal effect. Data we obtained from our *in vitro* time-kill study of glycopeptide antibiotics against our MRSA clinical isolates confirm their bacteriostatic effect and would explain the failure of glycopeptide therapy of *S. aureus* DAI cases studied.

Clearly, there is an important need for new antibiotic combinations which can enhance the bactericidal activity of vancomycin and teicoplanin. Fosfomycin is a phosphoenolpyruvate analogue produced by *Streptomyces* that irreversible inhibits enolpyruvate transferase, and prevents the formation of N-acetylmuramic acid, an essential element of the pepti-

doglycan cell wall. Five out of seven strains were characterized as resistant or intermediate to fosfomycin. However, since the major aim of this study was to evaluate the bactericidal activity of fosfomycin in combination with teicoplanin or vancomycin, we did not use a supplemented alfa-D-glucose-6-phosphate Mueller Hinton broth to test susceptibility to fosfomycin, a fact that probably explains the high rate of resistance of the strains studied. However, data obtained from the killing-curve method showed that the combination of fosfomycin and glycopeptides exhibits a bactericidal activity against MRSA strains isolated from patients with infections unresponsive to glycopeptide therapy already at a concentration of glycopeptide 1x MIC + fosfomycin 8 µg/mL.

These data are similar to a recent study by Grif *et al*, reporting a synergistic activity of fosfomycin with various antistaphylococcal agents [22]. However, it must be noted that the combination with vancomycin showed antagonism for two of seven isolates. This result is not confirmed in our study, showing an excellent synergistic activity of fosfomycin when combined with vancomycin. The mechanism responsible for this synergism has not yet been clarified: fosfomycin and glycopeptides have different binding sites on the peptidoglycan, a fact which may lead to more effective bactericidal activity; in addition, studying antibiotic susceptibility of four slime-producing isolates of *S. aureus*, Amorena et al. found that fosfomycin, followed by rifampicin, cefazolin, vancomycin and ciprofloxacin, significantly affects biofilm cell viability [23]. All these data seem to indicate a potential role of fosfomycin in combination with the glycopeptides for the treatment of severe MRSA DAI.

To our knowledge, there are no clinical studies evaluating the clinical benefit of the association between fosfomycin and glycopeptide. However, on the basis of our *in vitro* data, we recently treated two patients admitted in a Cardiac Surgery Intensive Care Unit who developed an MRSA bacteraemia unresponsive to vancomycin, and some cases of orthopaedic device-associated MRSA infections with a glycopeptide-fosfomycin combined therapy with achievement of a satisfactory clinical and microbiological response [3, 24, 25].

This combination might be useful not only in treating DAI but also in preventing the phenomenon of higher glycopeptide MICs. This indication remains to be proven by large series-based clinical studies.

Key words: Staphylococcus aureus, device-associated infections, glycopeptide failure, fosfomycin

SUMMARY

Fosfomycin is a molecule that inhibits the early stage of peptidoglycan synthesis and shows a broad-spectrum bactericidal activity against Gram-positive and Gram-negative bacteria. Using the Killing-curve method, we tested the *in vitro* bactericidal activity of fosfomycin alone or in combination with vancomycin or teicoplanin at a concentration of 8 µg/mL, that is easily achievable in serum at standard dosing regimens, against seven methicillin-resistant *Staphylococcus aureus* strains, isolated from patients with well documented device-associated infections unresponsive to or relapsing after glycopeptide therapy. MICs of vancomycin ranged from 1 to 4 µg/mL, MICs of teicoplanin from 2 to 8 µg/mL; MICs of fosfomycin were 8 µg/mL for two strains and >128 µg/mL for the remaining strains. The seven strains proved tolerant when tested for vancomycin and teicoplanin used alone at 2xMIC concentration. Fosfomycin was bactericidal (reduction of 2 log of the inoculum) only against the two susceptible strains. In all cases both vancomycin and teicoplanin in combination with fosfomycin developed bactericidal synergism already at a concentration of 1x MIC. If these results are confirmed by *in vivo* experiments, the combination of fosfomycin with glycopeptides might be useful for treating device-associated infections, and in preventing the phenomenon of increasing MICs for glycopeptides.

RIASSUNTO

La fosfomicina, un antibiotico ad ampio spettro attivo nei confronti di microrganismi Gram positivi e Gram negativi, esplica la propria attività battericida inibendo lo stadio iniziale della sintesi del peptidoglicano. Utilizzando la metodica delle curve di killing, abbiamo valutato l'attività battericida in vitro *di fosfomicina, da sola o in associazione con teicoplanina o vancomicina, ad una concentrazione facilmente ottenibile nel siero a seguito degli schemi terapeutici standard, e pari a 8 µg/mL, nei confronti di sette ceppi di* Staphylococcus aureus *meticillino-resistenti isolati da pazienti con infezione accertata associata a* devices *e non responsiva o recidivante a seguito di terapia con glicopeptidi. I valori di MIC di vancomicina erano compresi nel range 1-4 µg/mL, quelli di teicoplanina nel range 2-8 µg/mL; i valori di MIC di fo-sfomicina erano pari a 8 µg/mL per due ceppi mentre risultavano superiori a 128 µg/mL per i rimanenti ceppi. Allorquando esposti a concentrazioni di teicoplanina o vancomicina pari a 2xMIC, tutti i ceppi testati sono risultati tolleranti. Fosfomicina ha evidenziato un'attività battericida (riduzione dell'inoculo pari a 2 log) solo nei confronti dei due ceppi sensibili; viceversa l'associazione di fosfomicina con l'uno o l'altro dei due glicopeptidi ha evidenziato, per tutti i sette ceppi, un sinergismo battericida già a concentrazioni pari a 1xMIC. Se tali risultati* in vitro *trovassero conferma in esperimenti condotti* in vivo, *la terapia di associazione fosfomicina + glicopeptide potrebbe risultare di grande utilità nel trattamento delle infezioni associate a* devices, *oltre che nel prevenire l'aumento dei valori di MIC dei glicopeptidi.*

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