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Relationship of *In Vitro* Synergy and Treatment Outcome with Daptomycin plus Rifampin in Patients with Invasive Methicillin-Resistant *Staphylococcus aureus* Infections

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We report the findings of a study examining the relationship between *in vitro* daptomycin-rifampin synergy and the therapeutic outcome of 12 patients with complex deep methicillin-resistant *Staphylococcus aureus* (MRSA) infections treated for prolonged periods with this combination. Checkerboard synergy was found in nine cases and was 100% predictive of therapeutic success; absence of synergy was found in three cases, two of which were therapeutic failures ($P = 0.045$). No relationship was observed between synergy and outcome by time-kill assessment. Checkerboard synergy may predict clinical response to daptomycin plus rifampin for complex invasive MRSA infections requiring prolonged treatment.

Daptomycin is being increasingly used for the treatment of complicated Gram-positive infections, such as osteomyelitis and infections of prosthetic devices caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (1, 2), but the evidence for the effectiveness of combination regimens with daptomycin has been limited. In cases of primary treatment failure with *S. aureus* infections, rifampin has been recommended as an adjunctive therapeutic agent (3). Studies with *in vitro* and *in vivo* pharmacodynamic models of biofilm-associated infection support the use of daptomycin in combination with rifampin, where synergistic activity has been associated primarily with preventing the emergence of daptomycin-nonsusceptible strains (4–6). We have recently reported a high rate of success with daptomycin in combination with rifampin for treatment of complicated bone and joint infections (7), and in this study, we report the relationship between treatment outcome and *in vitro* synergy with complex deep MRSA infections.

Patients with invasive MRSA infections treated with daptomycin plus rifampin between 2005 and 2008 at the University of Wisconsin Hospital and Clinics in Madison, WI, were retrospectively reviewed. Clinical and microbiologic treatment outcomes were determined using standard definitions for osteomyelitis and nonosteomyelitis infections at clinical endpoints of 1 year and 4 weeks after discontinuation of therapy, respectively (1, 8), and compared to the results of *in vitro* synergy testing of the causative strain.

The MRSA isolates from 12 patients treated with the study combination were retrieved from the hospital clinical microbiology laboratory and analyzed. Daptomycin (Cubist, Lexington, MA) and rifampin (Sigma-Aldrich, St. Louis, MO) susceptibility testing was performed by broth microdilution on all isolates (9). Antibiotic susceptibility in biofilm was also determined since most patients had infection sources that commonly involve biofilm formation. The MIC and minimum biofilm eradication concentration (MBEC) were determined using a previously described transferable solid-phase pin-lid method (10). Synergy testing was evaluated by two methods: (i) checkerboard analysis with standard definitions of fractional inhibitory concentrations (FIC) (synergy, FIC $\leq$ 0.5; indifference, FIC of $>$0.5 but $\leq$4; antagonism, FIC $>$ 4) (11); (ii) 24-hour time-kill studies, with concentrations of daptomycin that are 0.5 to 4 times the organism MIC alone and concentrations of rifampin that are 0.5 times the MIC (synergy, $\geq$2 logs kill; additivity, 1 to 2 logs kill; indifference, 1 log kill to 1 log growth; antagonism, $\geq$1 log growth) (12). In this fashion, at least one of the antibiotics (rifampin) was present in a concentration with a minimal effect on growth when used alone in order to assess its interaction with daptomycin. All synergy studies were performed in duplicate. The relationship between *in vitro* synergy and clinical success was analyzed by Fisher's exact test using GraphPad Prism software (GraphPad, La Jolla, CA).

As expected, the MRSA infections in these patients were diverse and included five cases of refractory osteomyelitis, two infected postoperative surgical wounds, and one case each of prosthetic valve endocarditis, septic arthritis, a deep body cavity abscess, an infected prosthesis, and an infected hemodialysis catheter with bacteremia. Clinical cure (resolution of all signs and symptoms and no additional antibiotic therapy needed) or improvement (partial resolution of signs and symptoms) was documented in 10 of the 12 patients (83%). Only two patients failed with daptomycin-plus-rifampin therapy (Table 1).

The susceptibilities of the collected pathogens against daptomycin and vancomycin are listed in Table 1: all were susceptible to daptomycin (range, 0.13 to 1.0 μg/ml), and all but one were susceptible to rifampin (range, 0.03 to 8.0 μg/ml). In separate susceptibility experiments with biofilm cultures, up to an 8-fold increase in MIC was seen with daptomycin (the median MIC was 2.0 μg/ml in biofilm versus 0.25 μg/ml in planktonic cultures) while

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TABLE 1 Patient and isolate characteristics of cases treated with daptomycin plus rifampin for invasive methicillin-resistant *Staphylococcus aureus* infections[a]

Subject no.	Age and sex	Comorbidity(ies)	Infection type	MICs (μg/ml)	DAP (mg/kg of body wt) + RIF (mg) therapy	Clinical outcome	Synergy analysis	
							Checkerboard[b]	Time-kill[c]
1	66, F	DM, HTN	Osteomyelitis	DAP, 0.13; RIF, 0.03	4 mg/kg DAP q24h for 135 days; 600 mg RIF p.o. QD for 175 days	Cure	S	S
2	59, M	CKD, HTN, obesity	Osteomyelitis	DAP, 0.13; RIF, 0.03	6 mg/kg DAP q48h for 84 days; 600 mg RIF p.o. QD for 23 days	Cure	S	S
3	47, M	HTN, SLE, obesity, MRSA colonization	Postoperative wound	DAP, 0.13; RIF, 0.06	6 mg/kg DAP q48h for 90 days; 300 mg RIF p.o. q24h for 14 days	Cure	S	Add
4	31, M	ICU stay, ESRD HD, MRSA colonization	Endovascular/HD line	DAP, 0.25; RIF, 0.06	8 mg/kg DAP q48h for 90 days; 600 mg RIF p.o. q24h for 85 days	Cure	S	S
5	30, M	DM	Osteomyelitis	DAP, 0.25; RIF, 0.03	4 mg/kg DAP q24h for 46 days; 300 mg RIF p.o. q12h for 43 days	Improvement	I	I
6	22, M	Obesity	Septic arthritis	DAP, 0.13; RIF, 0.03	4 mg/kg DAP q24h for 68 days; 600 mg RIF p.o. q24h for 15 days	Cure	S	S
7	42, M	DM, CKD with transplant	Osteomyelitis	DAP, 0.13; RIF, 0.06	4 mg/kg DAP q24h for 42 days; 600 mg RIF p.o. q24h for 6 days	Cure	S	S
8	53, F	HTN	Joint prosthesis	DAP, 0.25; RIF, 0.03	4 mg/kg DAP q24h for 68 days; 600 mg RIF p.o. q24h for 39 days	Failure	I	Ant
9	53, F	ESRD HD, DM, HTN, obesity	Osteomyelitis	DAP, 0.25; RIF, 0.06	6 mg/kg DAP q48h for 43 days; 600 mg RIF p.o. q24h for 39 days	Cure	S	S
10	76, M	ESRD HD, DM, COPD, HTN	Postoperative wound	DAP, 1; RIF, 8	6 mg/kg DAP q48h for 43 days; 300 mg RIF p.o. q24h for 43 days	Cure	S	I
11	50, M	ICU stay, obesity	Prosthetic valve endocarditis	DAP, 0.25; RIF, 0.06	6 mg/kg DAP q24h for 100 days; 600 mg RIF p.o. q24h for 115 days	Cure	S	I
12	47, F	ICU stay	Deep abscess	DAP, 0.25; RIF, 0.03	6 mg/kg DAP q24h for 13 days; 600 mg RIF p.o. q12h for 83 days	Failure	I	I

[a] F, female; M, male; DM, diabetes mellitus; HTN, hypertension; CKD, chronic kidney disease; SLE, systemic lupus erythematosus; ICU, intensive care unit; ESRD, end-stage renal disease; HD, hemodialysis; COPD, chronic obstructive pulmonary disease; p.o., orally; q24h, every 24 h; QD, once daily; S, synergy; I, indifference; Add, additivity; Ant, antagonism.

[b] Classification based on fractional inhibitory concentration results.

[c] Classification based on time-kill curves with DAP at 0.5 times the MIC and RIF at 0.5 times the MIC.

there was no difference with rifampin (0.03 μg/ml in both planktonic and biofilm cultures). Similar fold changes occurred between cidal concentrations in planktonic (minimum bactericidal concentration [MBC]) and biofilm (MBEC) cultures (data not shown). The isolates from the two patients with microbiologic failure were susceptible to daptomycin.

By checkerboard assay, daptomycin and rifampin were synergistic against the infecting strain in nine (75%) of the 12 cases; indifference was found in the remaining three MRSA cases, and no antagonism was detected. In a correlation of clinical outcome to the results of synergy testing by checkerboard with the infecting strains, checkerboard synergy was found in the nine cases and was 100% predictive of therapeutic success; absence of synergy was found in three cases, two of which were therapeutic failures ($P = 0.046$; Fisher's exact test).

Synergy was also examined by time-kill curves. The representative results from a single MRSA strain are displayed in Fig. 1. At exposures of 0.5 times the MIC of daptomycin and rifampin, six of the 12 strains tested exhibited synergy while the remaining strains had indifference (4), additivity (1), or antagonism (1). However, when daptomycin concentrations were increased to 4 times the MIC, with rifampin held at 0.5 times the MIC, no synergy was found. At daptomycin concentrations of 4 times the MIC, 11 of the 12 isolates showed indifference while one isolate exhibited antagonism. In the first 4 h of exposure, antagonism was often present with high daptomycin concentrations when combined with rifampin, a finding which is consistent with other reports with similar concentration ratios (5, 13). There was no correlation between patient outcome and *in vitro* synergy by time-kill assay.

Daptomycin in combination with adjunctive antibiotics, such as rifampin, aminoglycosides, or co-trimoxazole, may improve antimicrobial killing or inhibit the emergence of resistance compared to daptomycin alone (5, 14). New Infectious Diseases Society of America (IDSA) guidelines for the treatment of prosthetic joint infections recommend combining rifampin with primary therapy (15), but published clinical experiences with daptomycin combined with rifampin have been very limited. We have recently reported an 86% rate of success at 3 to 5 years in a cohort of 29 patients with osteomyelitis or infected total joint prostheses, most of whom had failed conventional therapy, usually with vancomycin (7).

Previous *in vitro* synergy studies with daptomycin and rifampin have demonstrated an additive effect in laboratory MRSA strains (4, 7). This combination in our study was synergistic or indifferent in all of our strains in the checkerboard assay, while no synergy was found in the majority of the strains with the time-kill technique. Our study is unique in that we correlate the findings of *in vitro* synergy testing with clinical treatment outcome in patients with complex deep MRSA infections. Daptomycin plus rifampin showed *in vitro* synergy by checkerboard testing in 75% of the isolates, which correlated 100% with clinical cure in patients with complex MRSA infections treated with this combination. We hypothesize that the checkerboard method may better assess the range of antibiotic concentration ratios that occurs in sequestered infection sites in which antibiotic penetration may be delayed and highly variable. This method may not be ideal to predict outcomes of acute bloodstream infections, in which antibiotic pharmacokinetics can be more directly estimated with static or dynamic pharmacokinetic (PK)/pharmacodynamic (PD) models. Our study is limited by a relatively small number of observations and requires

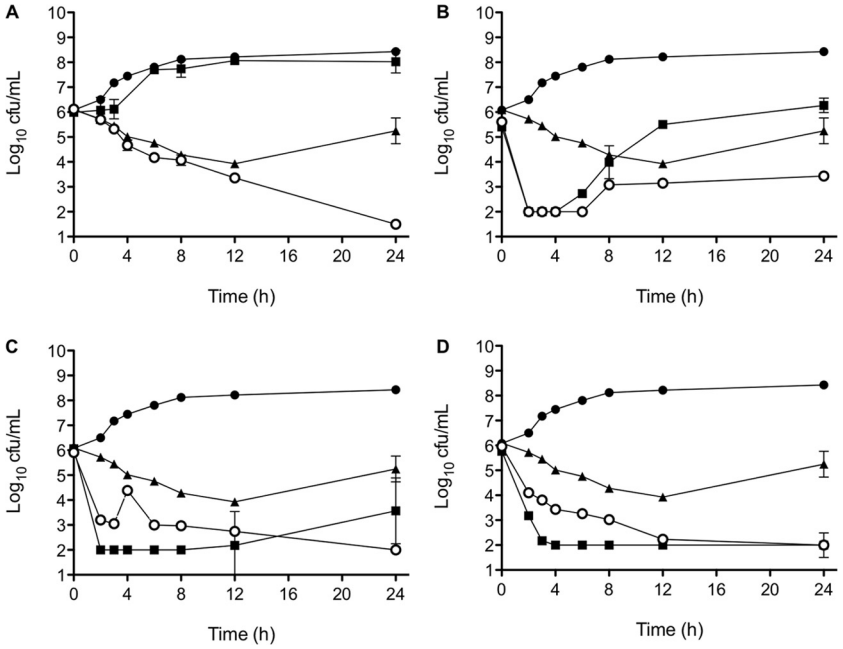

FIG 1 Results of the killing curve study with daptomycin (DAP) and rifampin (RIF) activity alone and in combination against a single MRSA strain from a subject. (A) DAP at 0.5 times the MIC and RIF at 0.5 times the MIC; (B) DAP at 1 times the MIC and RIF at 0.5 times the MIC; (C) DAP at 2 times the MIC and RIF at 0.5 times the MIC; (D) DAP at 4 times the MIC and RIF at 0.5 times the MIC. Black circles, growth control; squares, DAP; triangles, RIF; white circles, DAP plus RIF. The analysis shows synergy with low concentrations of both DAP and RIF but early antagonism as DAP concentrations increased.

further validation, but the checkerboard method for demonstrating *in vitro* synergy between daptomycin and rifampin may be a reliable predictor of therapeutic success in complex MRSA infections treated with this novel combination.

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