Title

Combinations of Daptomycin plus Ceftriaxone, but Not Ascending Daptomycin Dose-Regimens, Are Effective in Experimental Endocarditis Caused by Streptococcus mitis-oralis Strains: Target Tissue Clearances and Prevention of Emergence of Daptomycin-Resis...

Journal

Antimicrobial Agents and Chemotherapy, 67(4)

Authors

Abdelhady, Wessam
Elsayed, Ahmed
Lapitan, Christian
et al.

Publication Date

2023-04-18

DOI

10.1128/aac.01472-22

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

CLINICAL THERAPEUTICS

Combinations of Daptomycin plus Ceftriaxone, but Not Ascending Daptomycin Dose-Regimens, Are Effective in Experimental Endocarditis Caused by *Streptococcus mitis-oralis* Strains: Target Tissue Clearances and Prevention of Emergence of Daptomycin-Resistance

Nagendra N. Mishra,[a,b] Wessam Abdelhady,[a] Ahmed M. Elsayed,[a] Christian Lapitan,[a] Richard A. Proctor,[c,d] Michael J. Rybak,[e,f] Jose M. Miro,[g,h] Arnold S. Bayer[a,b]

[a]Division of Infectious Diseases, The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, California, USA
[b]The David Geffen School of Medicine, University of California, Los Angeles, California, USA
[c]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA
[d]Department of Medical Microbiology & Immunology, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA
[e]Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences, Detroit, Michigan, USA
[f]School of Medicine, Wayne State University, Detroit, Michigan, USA
[g]Hospital Clinic, Institut d' Investigacions Biomèdiques August Pi, iSunyer, University of Barcelona, Barcelona, Spain
[h]CIBERINFEC, Instituto de Salud Carlos III, Madrid, Spain

ABSTRACT The *Streptococcus mitis-oralis* subgroup of the viridans group streptococci (VGS) are the most common cause of infective endocarditis (IE) in many parts of the world. These organisms are frequently resistant *in vitro* to standard β-lactams (e.g., penicillin; ceftriaxone [CRO]), and have the notable capacity for rapidly developing high-level and durable daptomycin resistance (DAP-R) during exposures *in vitro*, *ex vivo*, and *in vivo*. In this study, we used 2 prototypic DAP-susceptible (DAP-S) *S. mitis-oralis* strains (351; and SF100), which both evolved stable, high-level DAP-R *in vitro* within 1 to 3 days of DAP passage (5 to 20 μg/mL DAP). Of note, the combination of DAP + CRO prevented this rapid emergence of DAP-R in both strains during *in vitro* passage. The experimental rabbit IE model was then employed to quantify both the clearance of these strains from multiple target tissues, as well as the emergence of DAP-R *in vivo* under the following treatment conditions: (i) ascending DAP-alone dose-strategies encompassing human standard-dose and high-dose-regimens; and (ii) combinations of DAP + CRO on these same metrics. Ascending DAP-alone dose-regimens (4 to 18 mg/kg/d) were relatively ineffective at either reducing target organ bioburdens or preventing emergence of DAP-R *in vivo*. In contrast, the combination of DAP (4 or 8 mg/kg/d) + CRO was effective at clearing both strains from multiple target tissues (often with sterilization of bio-burdens in such organs), as well as preventing the emergence of DAP-R. In patients with serious *S. mitis-oralis* infections such as IE, especially caused by strains exhibiting intrinsic β-lactam resistance, initial therapy with combinations of DAP + CRO may be warranted.

KEYWORDS daptomycin, ceftriaxone, endocarditis, *S. mitis-oralis*

The *Streptococcus mitis-oralis* subgroup of the viridans streptococci includes a cadre of oropharyngeal organisms, including *S. mitis*, *S. oralis*, *S. gordonii*, and *S. parasanguinis* (1–4). This subgroup represents an important human pathogen group, being the most common overall cause of infective endocarditis (IE) in the developing world (4–6), and the predominant cause of the "streptococcal toxic shock syndrome" in

Address correspondence to Nagendra N. Mishra, nmishra@lundquist.org.

The authors declare no conflict of interest.

Received 2 November 2022
Returned for modification 5 December 2022
Accepted 2 February 2023
Published 6 March 2023

immunocompromised patients (5–9). The *in vitro* antimicrobial susceptibility profiles of *S. mitis-oralis* strains often present therapeutic conundrums, as a high per cent of such isolates are resistant to penicillins and/or cephalosporins (including third generation agents such as ceftriaxone [CRO]) (10–14). Interestingly, 'vancomycin tolerance' in *S. mitis* has also been described (9). These scenarios usually raise the notion of using daptomycin (DAP) as alternative therapy for such infections. However, several recent studies have confirmed the unique capacity of *S. mitis-oralis* strains to rapidly develop high-level and durable DAP resistance (DAP-R) when exposed to this agent *in vitro*, *ex vivo* in simulated IE models, and *in vivo* in experimental IE (4, 15–17). Thus, in 2013, Garcia-de-la-Maria et al. (4) first documented that a substantial proportion of *S. mitis-oralis* clinical bloodstream isolates (>25%) had the notable property to rapidly (within 48 h *in vitro* exposures) evolve high-level, stable DAP-R. In addition, this research group demonstrated that such high-level DAP-R could also emerge *in vivo* during DAP-alone therapy of experimental IE (4). Further, they showed that combination therapy with DAP + gentamicin was able to synergistically clear DAP-susceptible (DAP-S) parental strains from cardiac vegetations in this model, while forestalling evolution of DAP-R subpopulations during therapy (4). Furthermore, *in vitro* and *ex vivo* studies featuring DAP combination regimens have shown the ability of several regimens to either synergistically kill *S. mitis-oralis* strains and/or prevent the emergence of DAP-R during DAP exposures. Such regimens have included DAP + either gentamicin, CRO, ceftaroline, or trimethoprim-sulfamethoxazole (4, 15–17). Of note, the combination therapy outcomes *in vitro* and *ex vivo* were most impressive with DAP + either ceftaroline or CRO.

This study was designed to utilize the experimental IE model caused by 2 distinct and well-characterized DAP-S *S. mitis-oralis* strains, in order to answer 2 key questions: (i) could ascending DAP-alone dose-regimens that encompassed "high-dose" human dose-regimens increase target organ clearances (vegetations, kidneys, and spleen), and also prevent emergence of DAP-R; and (ii) could DAP combination regimens with CRO achieve these same goals? The results of these investigations clearly showed the inability of increasing DAP-alone dosing strategies, on the one hand, to exert such salutary impacts on *in vivo* efficacy and DAP-R emergence, while DAP + CRO regimens were quite effective in achieving both such outcomes.

The term "DAP resistance (DAP-R)" (instead of "DAP-nonsusceptibility") is used throughout the manuscript for ease of presentation. The term "ascending" DAP dose-regimens indicates increasing doses used within separate treatment groups of animals (not within the same animal treatment groups).

RESULTS

MICs. The previously published DAP and CRO MICs of the DAP-S 351 and SF100 strains were reconfirmed. The 351 and SF100 strains were susceptible to DAP, each exhibiting DAP MICs of 0.5 μg/mL, however, the 351 strain was resistant to CRO (MIC = 8 μg/mL), while the SF100 strain was susceptible to CRO (MIC = 0.125 μg/mL) (16).

Prevention of emergence of DAP-R during *in vitro* passage studies. We determined the MICs of bacterial samples daily at 24 h intervals during a 10 day passage period in DAP-alone or DAP + CRO to assess potential prevention of evolution of DAP-R (Table 1 and 2). Daily passage of each DAP-S strain in distinct concentrations (5, 10, and 20 μg/mL) of DAP-alone resulted in rapid emergence of high-level DAP-R populations by the first 1 to 2 days of passage (Table 1 and 2). In contrast, when the DAP-S 351 and SF100 parental strains were passaged in DAP + CRO, DAP MICs were maintained at relatively low levels (1 to 4 μg/mL), even at higher DAP dose-regimens (Table 1 and 2). None of the postpassaged strains were resistant to CRO. In addition, 351-derived and SF100-derived day 10 (D10) DAP-R passage strains exhibited stable DAP MICs when passaged for 5 additional days in antibiotic-free media (data not shown) (18–20). Any postpassaged strains re-passaged in antibiotic-free media exhibited DAP MIC values $\geq$ 2 μg/mL were considered DAP-R.

Experimental IE. For the DAP-S parental strain 351, although treatments with ascending doses of DAP-alone (4 to 18 mg/kg/d) statistically reduced counts in vegetations and other

TABLE 1 *In vitro* serial passaging of 351 *S. mitis-oralis* strain in combination of distinct concentrations of DAP with CRO versus DAP alone

Distinct concentrations (μg/mL) of DAP alone and DAP + CRO	DAP MICs (μg/mL) of strain 351 passaged in ascending concentrations of DAP alone or DAP + CRO on distinct days									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
DAP (5)	4	256	256	256	256	256	256	256	256	256
DAP (10)	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
DAP (20)	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
DAP (5) + CRO (1)	2	4	4	4	4	4	4	4	4	4
DAP (10) + CRO (1)	2	2	2	2	2	2	2	2	2	2
DAP (20) + CRO (1)	2	2	2	2	2	2	2	2	2	2

target tissues compared to untreated controls, the bioburden reductions were modest, at best (Table 3). Moreover, the majority of vegetations now contained high proportions of DAP-R subpopulations after 4 days of therapy, even at the highest DAP-alone dose-regimens.

CRO alone was also relatively ineffective at bacterial clearances in any target tissues (Table 3); no CRO-R subpopulations were detected. In contrast, combination therapy with DAP + CRO was highly effective at clearing strain 351 from multiple target tissues in a DAP dose-dependent manner (DAP 4 mg/kg/d versus 8 mg/kg/d) (Table 3). At the DAP 4 mg/kg dose + CRO, there were essentially no intravegetation DAP-R subpopulations, despite moderate residual CFU/g bioburdens persisting in this target tissue (Table 3). Of note, the DAP 8 mg/kg dose-regimen + CRO sterilized all target tissues (Table 3).

Treatment outcomes for DAP-S parental strain, SF100, were somewhat similar to strain 351 (Table 4), with several important differences. As with strain 351, ascending DAP-alone dosing exerted very modest impacts on significantly reducing target tissue counts; in addition, substantial proportions of the vegetation bioburdens emerged as DAP-R in these DAP-alone treatment groups. As opposed to strain 351, however, CRO alone was effective at significantly (albeit modestly) reducing vegetation bioburdens of strain SF100 (Table 4); moreover, CRO alone sterilized most kidney and spleen infection (Table 4). As seen with strain 351, the combination of DAP + CRO further cleared vegetations of strain SF100 in a DAP dose-dependent manner (Table 4). Both DAP + CRO regimens were highly effective at significantly reducing tissue bioburdens within kidneys and spleens, sterilizing all lesions in both target organs (Table 4).

DISCUSSION

These investigations assessed if either increasing DAP-alone dosing strategies or DAP + CRO combinations could improve *in vivo* efficacy and avoid DAP-R emergence in experimental *S. mitis-oralis* IE. The notion of utilizing DAP-based antibiotic combination regimens for *S. mitis-oralis* therapy has been previously pursued both *in vitro* and *ex vivo* (in simulated IE models) (15, 16). Zapata et al. (15) recently evaluated a broad range of DAP combination regimens for their abilities to both synergistically kill and/or prevent emergence of DAP-R during passage, utilizing the same DAP-S parental strain (351) employed in this study. The combination antibiotics included: DAP + either gentamicin, rifampin, trimethoprim-sulfamethoxazole, imipenem, ceftaroline, tedizolid, and linezolid. Only the combination of DAP + either

TABLE 2 *In vitro* serial passaging of SF100 *S. mitis-oralis* strain in combination of distinct concentrations of DAP with CRO versus DAP alone

Distinct concentrations (μg/mL) of DAP alone and DAP + CRO	DAP MICs (μg/mL) of strain 351 passaged in ascending concentrations of DAP alone or DAP + CRO on distinct days									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
DAP (5)	2	2	>256	>256	>256	>256	>256	>256	>256	>256
DAP (10)	2	>256	>256	>256	>256	>256	>256	>256	>256	>256
DAP (20)	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256
DAP (5) + CRO (0.0156)	1	1	1	1	4	4	4	4	4	4
DAP (10) + CRO (0.0156)	1	1	1	1	2	2	2	2	2	2
DAP (20) + CRO (0.0156)	0.5	4	4	4	4	4	4	4	4	4

TABLE 3 Treatment of 351 *S. mitis-oralis* strain with ascending dosages of DAP in combination of CRO versus DAP and CRO alone in *in vivo* IE model[a]

Treatment	Vegetation [sterile] (/= Growth on DAP 2 mg/L plates)[b]	Kidney	Spleen
Untreated controls (7)	8.49 ± 0.65	5.27 ± 0.71	5.26 ± 0.36
DAP 4 mg/kg i.v. once daily × 4 d (7)	7.66 ± 0.87/6.14 ± 1.47[c]	4.16 ± 0.78[c]	4.20 ± 0.99[c]
DAP 6 mg/kg (7)	7.43 ± 1.06[d]/6.09 ± 0.93[d]	3.90 ± 0.67[d]	5.06 ± 1.22
DAP 8 mg/kg (6)	8.24 ± 0.82/6.50 ± 1.53[e]	4.71 ± 0.91	4.94 ± 0.80
DAP 10 mg/kg (6)	7.50 ± 1.08/5.53 ± 0.51[f]	4.18 ± 0.49[f]	4.13 ± 0.52[f]
DAP 12 mg/kg (7)	7.14 ± 1.04[g]/6.63 ± 1.04[g]	3.96 ± 0.59[g]	3.79 ± 0.53[g]
DAP 18 mg/kg (6)	8.12 ± 0.79/7.79 ± 0.83	3.94 ± 0.51	4.99 ± 0.30
CRO 40 mg/kg i.v. twice daily × 4 d (7)	7.81 ± 0.65	3.94 ± 0.51[h]	4.53 ± 1.04
DAP (4 mg/kg) + CRO (6)	5.51 ± 1.18[i,j,k] /0.62 ± 0.07[i,j,k]	1.93 ± 0.72[i,j,k]	2.49 ± 0.48[i,j,k]
DAP (8 mg/kg) + CRO (6)	0.62 ± 0.07[l,m,n] /0.62 ± 0.07 [6/6][l,m,n]	0.69 ± 0.08 [6/6][l,m,n]	0.76 ± 0.13 [6/6][l,m,n]

[a]Statistical differences for Untreated controls versus DAP 4, 6, 8, 10, 12 mg/kg; Untreated controls versus CRO alone; Untreated controls versus DAP + CRO; combination of DAP + CRO versus DAP alone; combination of DAP + CRO versus CRO alone were determined by Student's *t* test.
[b]These designations define the number of sterile target tissues/number sampled (e.g., 6/6 = all 6 target tissues were sterilized), as well as the proportion of vegetations having DAP-R subpopulations as defined by growth on DAP plates (2 mg/L).
[c]$P < 0.05$ untreated controls versus DAP 4 mg/kg.
[d]$P < 0.05$ untreated controls versus DAP 6 mg/kg.
[e]$P < 0.05$ untreated controls versus DAP 8 mg/kg.
[f]$P < 0.01$ untreated controls versus DAP 10 mg/kg.
[g]$P < 0.05$ untreated controls versus DAP 12 mg/kg.
[h]$P < 0.005$ untreated controls versus CRO alone.
[i]$P < 0.005$ DAP 4 mg/kg + CRO versus DAP 4 mg/kg.
[j]$P < 0.005$ untreated controls versus DAP 4 mg/kg + CRO.
[k]$P < 0.01$ DAP 4 mg/kg + CRO.
[l]$P < 0.0001$ DAP 8 mg/kg + CRO. Parenthesis () = number of rabbits.
[m]$P < 0.0001$ untreated controls versus DAP 8 mg/kg + CRO.
[n]$P < 0.0005$ DAP 8 mg/kg + CRO versus DAP 8 mg/kg.

gentamicin or ceftaroline were both bactericidal and synergistic in early time-kill curve interactions. Of note, the combinations of DAP + gentamicin, ceftaroline, or trimethoprim-sulfamethoxazole prevented DAP-R emergence during serial passage experiments. Yim et al. (17) 20 used the same 2 DAP-S strains employed in this study (351 and SF100) in their simulated IE model *ex vivo*. As opposed to the *in vitro* results noted above, the addition of gentamicin to DAP (given in human-simulating PK-PD dose-regimens) in this *ex vivo* model yielded neither synergistic nor bactericidal interactions against either *S. mitis-oralis* strain; moreover, this regimen did not forestall the evolution of DAP-R over the 96 h exposure period in this model. Of interest, combinations of DAP + either ceftaroline or CRO accomplished both these latter desired metric outcomes. This *ex vivo* data led us to examine the combined efficacy of DAP plus CRO in the experimental IE model *in vivo* in this investigation.

Several notable observations emerged from this study. First, we hypothesized that DAP-alone dose-escalation, in the presence of an intact host defense system microenvironment (e.g., antimicrobial peptides, macrophages-neutrophils, etc.), might: (i) synergize well to clear *S. mitis-oralis* from infected target tissues; and (ii) override the propensity for *S. mitis-oralis* strains to develop DAP-R. However, this notion proved to be false. This speaks to the rapid adaptive capacity of these organisms to circumvent DAP exposures. We have previously defined the mechanism(s) driving DAP-R evolution in *S. mitis-oralis*; these are strain-specific and multifactorial, involving rapid and major phenotypic, metabolic, and genotypic adaptations, including: (i) reduction in the major membrane phospholipids, phosphatidylglycerol and cardiolipin (both of which participate in initial interactions of DAP with target bacterial membranes, and which also "guide" DAP to the divisome site-of-action for DAP) (21, 22). These perturbations are accompanied by loss-of-function mutations in key genes within the cardiolipin biosynthetic pathway, including *pgsA* and *cdsA* (21, 22); (ii) preferential accumulation of DAP in selected bacterial cells within streptococcal chains, presumably sequestering the drug in a minority of the subpopulation, while "protecting" the rest of the bacterial population from its bactericidal activity (18); (iii) exaggeration of the fermentative glycolytic pathway in these organisms, which lack a traditional TCA cycle, reducing generation of biometabolic intermediates and ATP (18, 19); and (iv) modifications of the

TABLE 4 Treatment of SF100 *S. mitis-oralis* strain with ascending dosages of DAP in combination of CRO versus DAP and CRO alone in *in vivo* IE model[a]

Treatment (no. of animals)	Vegetation [sterile] (/ = Growth on DAP 2 mg/L plates)[b]	Kidney	Spleen
Untreated Controls (8)	7.93 ± 0.57	4.58 ± 0.52	4.70 ± 0.85
DAP 4 mg/kg i.v. once daily × 4 days (6)	7.80 ± 0.81/1.00 ± 0.06[c]	3.50 ± 0.49[c]	3.54 ± 0.28[c]
DAP 6 mg/kg (6)	7.42 ± 1.08/3.53 ± 2.63[d]	4.51 ± 0.63	4.45 ± 0.49
DAP 8 mg/kg (6)	7.69 ± 0.75/4.30 ± 2.78[e]	3.24 ± 0.41[e]	3.40 ± 0.90[e]
DAP 10 mg/kg (6)	7.51 ± 0.80/5.72 ± 1.98[f]	3.41 ± 0.63[f]	3.64 ± 0.84[f]
DAP 12 mg/kg (6)	7.01 ± 1.07/4.28 ± 2.57[g]	3.86 ± 0.76	3.64 ± 0.79[g]
DAP 18 mg/kg (6)	7.25 ± 0.84/5.74 ± 1.28	4.42 ± 0.78	4.77 ± 0.45
CRO 40 mg/kg i.v. once daily × 4 days (6)	5.68 ± 0.93 [0/6][h]	0.95 ± 0.41 [5/6][h]	1.13 ± 0.48 [5/6][h]
DAP (4 mg/kg) + CRO (6)	3.75 ± 1.59 [1/6][i,j,k]	0.76 ± 0.07 [6/6][i,j]	0.89 ± 0.12 [6/6][i,j]
DAP (8 mg/kg) + CRO (7)	0.89 ± 0.19 [7/7][l,m,n]	0.92 ± 0.11 [7/7][m,n]	0.92 ± 0.08 [7/7][m,n]

[a]Statistical differences for Untreated controls versus DAP 4, 6, 8, 10, 12 mg/kg; Untreated controls versus CRO alone; Untreated controls versus DAP + CRO; combination of DAP + CRO versus DAP alone; combination of DAP + CRO versus CRO alone were determined by Student's *t* test.
[b]These designations define the number of sterile target tissues/number sampled (e.g., 6/6 = all 6 target tissues were sterilized), as well as the proportion of vegetations having DAP-R subpopulations as defined by growth on DAP plates (2 mg/L).
[c]$P < 0.01$ untreated controls versus DAP 4 mg/kg.
[d]$P < 0.01$ untreated controls versus DAP 6 mg/kg.
[e]$P < 0.05$ untreated controls versus DAP 8 mg/kg.
[f]$P < 0.05$ untreated controls versus DAP 10 mg/kg.
[g]$P < 0.05$ untreated controls versus DAP 12 mg/kg.
[h]$P < 0.001$ untreated controls versus CRO alone.
[i]$P < 0.001$ DAP 4 mg/kg + CRO versus DAP 4 mg/kg.
[j]$P < 0.001$ untreated controls versus DAP 4 mg/kg + CRO.
[k]$P < 0.05$ DAP 4 mg/kg + CRO.
[l]$P < 0.0001$ DAP 8 mg/kg + CRO. Parenthesis () = number of rabbits.
[m]$P < 0.00001$ untreated controls versus DAP 8 mg/kg + CRO.
[n]$P < 0.01$ DAP 8 mg/kg + CRO versus DAP 8 mg/kg.

organism's membrane and cell surface phenotypes, including enhanced fluidity and surface charge, both of which can negatively influence DAP's bactericidal mechanisms (17, 21, 22). Clearly, these adaptations occur very rapidly during DAP-alone exposures in selected DAP-S *S. mitis-oralis* strains that appear to be "destined" or "predisposed" to evolve DAP-R, and are not preventable by merely increasing the initial DAP-alone exposure concentrations.

Second, as was seen previously *in vitro* and *ex vivo* (15, 16, 22), combining selected agents with DAP can provide an outcome advantage *viz-a-viz* both streptococcal clearances and prevention of DAP-R. These combinatorial impacts were recapitulated with the DAP + CRO combinations used in the experimental IE model. Notably, this combination was highly effective at clearing all target tissues of both *S. mitis-oralis* parental strains, and also rendered most of these organs (e.g., vegetations, spleen, kidney) culture-negative. This combination also prevented the rapid emergence of DAP-R or CRO-R subpopulations within target organs in this model. It should be noted that the DAP + CRO efficacy in this model was most prominent in clearing strain SF100 (which was CRO-susceptible *in vitro*) from multiple target tissues.

Lastly, as alluded to above, both of the study strains (351 and SF100) are organisms that appear "destined" to rapidly evolve high-level and stable DAP-R *in vitro*, *ex vivo*, and *in vivo* during DAP-alone exposures. This latter phenotype occurs in at least 25% of *S. mitis-oralis* strains (4). We are currently examining those phenotypic, metabolic, and genotypic characteristics that distinguish *S. mitis-oralis* strains that appear predisposed to evolve DAP-R (or not). If a specific and predictive set of phenotypic or genotypic biomarkers can be identified up-front, that correlate with risk of eventual evolution of DAP-R, this could be a major advance forward in precision selection of agents to treat such infections.

Our study had several limitations. It was limited to only 2 prototype *S. mitis-oralis* strains; additional *S. mitis-oralis* strains need to be tested in this same model, especially those that are apparently "not predisposed" to evolve DAP-R *in vitro* (which represent the majority of *S. mitis-oralis* strains [4]). In addition, follow-up studies in this same model should also examine higher CRO alone dose-regimens to see if there is an optimal strategy to eradicate multiple *S. mitis-oralis* strains from all target tissues with such therapy.

In conclusion, the combination of DAP + CRO was effective at clearing both *S. mitis-oralis* strains *in vivo* from multiple target tissues, as well as in preventing the emergence

of DAP-R. In patients with *S. mitis-oralis* IE, especially caused by strains exhibiting intrinsic β-lactam resistance, initial therapy with combinations of DAP + CRO may be warranted.

MATERIALS AND METHODS

Bacterial strains. The well-characterized DAP-S *S. mitis-oralis* strains, 351 and SF100, were used in this study (4, 15–17, 21). Both strains were initially isolated from the bloodstream of patients with IE without prior DAP exposures. Strain SF100 was kindly provided by Dr. Paul Sullam (San Francisco, CA). The penicillin MIC for the 351 strain has been previously reported by both Etest and broth microdilution assays as 8 μg/mL (penicillin-resistant) 4); the penicillin MIC for strain SF100 was 0.25 μg/mL (penicillin-susceptible). In addition, both 351 and SF100 strains are ceftaroline-susceptible, as tested previously (15, 16).

MICs. DAP was purchased from Merck & Co., Inc. DAP MIC testing was performed by the CLSI-recommended broth microdilution techniques, with 50 μg/mL of $CaCl_2$ added to BHI broth (Difco). Also, BHI agar supplemented with 5% lysed horse blood (Difco) was used for quantitative agar plate colony counts. DAP MICs were also determined in parallel by standard Etest (bioMérieux) on Mueller-Hinton agar (MHA) plates supplemented with 50 μg/mL of $CaCl_2$ (Difco Laboratories). There are no formal CLSI DAP breakpoints against VGS strains; however, streptococcal strains with DAP MICs $\geq$ 2 μg/mL are considered as DAP-R (4, 15–17, 21–23). The MICs of CRO against *S. mitis-oralis* strains 351 and SF100 were determined by broth microdilution methods; VGS strains with CRO MICs $\geq$ 4 μg/mL are defined as CRO-resistant. A minimum of 3 independent broth microdilution-based MICs were carried out on different days for all agents, with the average MIC reported.

Prevention of emergence of DAP a resistance *in vitro*. We evaluated the impacts of longer-term DAP +/− CRO exposures of the 2 DAP-S *S. mitis-oralis* strains on the potential emergence and stability of DAP-R (15–19, 21–23). For these latter studies, the DAP-S parental strains 351 and SF100 were cultured overnight in BHI broth. The initial inoculum of strain SF100 and 351 (OD_{600} = 1.00 = ~108 CFU/mL) were exposed to 5, 10, or 20 μg/mL of DAP in BHI + 50 μg/mL $CaCl_2$ alone or with the combination of CRO (1/4 $\times$ MICs of each strain); surviving colonies were serially passaged for a 10-day period under the same conditions (15). Surviving colonies after each day's passage were stored at −80°C for subsequent MIC testing. The stability of DAP-R was determined by growing such strains for 5 additional days in antibiotic-free media and repeating the DAP MICs as above.

The DAP and CRO MICs of strains 351 and SF100 following serial passage in DAP alone versus combination of DAP + CRO regimens were determined by broth dilution supplemented with 5% lysed horse blood (as recommended by CLSI for *S. mitis-oralis*) (15). We also assessed the stability of DAP-R isolates that appeared during the 10-day passage period. Individual postpassage isolates were again serially passaged for 5 days in antibiotic-free BHI media (15). The DAP and CRO MICs of these antibiotic-free postpassage isolates were evaluated by broth dilution assay (15); A minimum of 3 independent experiments were performed on separate days to analyze the MIC data.

Experimental IE model. To verify the *in vivo* translatability of the relationship between emergence of DAP-R during either ascending DAP-alone or combined DAP-CRO treatment of *S. mitis-oralis* strains *in vitro*, the well-characterized rabbit model of indwelling catheter-induced aortic valve IE was used (4). Rabbits were i.v. infected at 48 h after catheter placement with: 1×10^7 CFU/animal for strain 351; and 8×10^6 CFU/animal for strain SF100. These inocula represent the ID_{95} for inducing IE and establishing infection in key extracardiac target tissues (kidneys and spleen) as determined by extensive pilot experiments for each strain. At 24 h postinfection, animals were randomized into either an untreated control group (sacrificed at this time point as a therapeutic baseline) or the following antibiotic therapy groups: (i) DAP-alone, given in separate groups at ascending doses of 4 to 18 mg/kg/d i.v. for 4 days. These dose-regimens encompass subtherapeutic to human-mimicking therapeutic doses in humans; for example, 12 to 18 mg/kg DAP dosing regimens equate to 6 to 10 mg/kg human dose PK-PD, respectively; this human dose-range encompasses the current FDA-approved dose-regimen for serious infections (6 mg/kg/d), as well as the human "high-dose" DAP regimens (10 mg/kg/d) (17, 20, 21); (ii) CRO alone (40 mg/kg; bid, iv) has been successfully used in prior experimental streptococcal IE studies (16, 17), and mimics human PK-PD (16, 17); and (iii) the combination of DAP (4 or 8 mg/kg/d) plus CRO (40 mg/kg/d iv, bid). Serum levels of DAP and CRO were not assessed, as these have been previously published in this model using similar dose-regimens (20, 24).

In all studies, at 24 h after the last antibiotic treatment (to circumvent any antibiotic carryover effects), animals were sacrificed, and their cardiac vegetations, kidneys, and spleen were aseptically removed and quantitatively cultured on TSA plates, as previously described (22). Counts were expressed as mean $\log_{10}$ CFU per gram of tissue ($\pm$SD). The limit of detection in target organ cultures in this model, based on average target tissue weights, is $\leq$2 $\log_{10}$ CFU/g. All culture-negative vegetations, although designated as "sterile," were still assigned a limit-of-detection $\log_{10}$ CFU/g designation based on their weights, for statistical purposes, to enable comparisons between groups. This is a standard procedure used in this model. To assess for *in vivo* development of DAP or CRO resistances in cardiac vegetations during treatments, serial dilutions of these homogenates were parallel-plated on either DAP-containing agar plates (2 μg/mL) or CRO-containing agar plates (8 μg/mL), depending on the specific treatment groups. Only cardiac vegetations were parallel-plated for emergence of antibiotic resistances, given the high organ bioburdens usually achieved in this infection site. Thus, any potential emergence of DAP-R or CRO-R would be most likely to occur in this anatomic site.

Statistics. Data were presented, unless otherwise indicated, as the sample mean $\log_{10}$ CFU/g tissue $\pm$SD. All head-to-head statistical comparisons were made using the unpaired Student's *t* test. *P* values $<$ 0.05 were considered statistically significant.

Study approval. Female, New Zealand White rabbits weighing 2.2 to 2.5 kg were used in all animal studies (Irish Farms; Riverside, CA). Rabbits were maintained in accordance with the American Association

for Accreditation of Laboratory Animal Care criteria. The Institutional Animal Care and Use Committee of the Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center approved all animal study protocols.

ACKNOWLEDGMENTS

This study was supported, in part, by grants from the U.S. National Institutes of Health 5-R01-AI-130056 (to A.S.B.) and R01 AI-121400 (to M.J.R.). N.N.M. was supported by The Lundquist Institute for Biomedical Innovation at Harbor-UCLA by an intramural research grant (#531604-01-01).

We thank Paul Sullam (UCSF; San Francisco, CA) for his many helpful suggestions on this overall project.

Jose M. Miro has received a Personal 80:20 Research Grant from Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

REFERENCES

1. Holland TL, Bayer AS, Fowler VG. 2020. Endocarditis and intravascular infections, p 1068–1108. *In* Mandell GL, Bennett JE. (ed), Principles and practices of infectious diseases, 9th ed; Elsevier, Philadelphia, PA, USA.
2. Ahmed R, Hassall T, Morland B, Gray J. 2003. Viridans streptococcus bacteremia in children on chemotherapy for cancer: an underestimated problem. Pediatr Hematol Oncol 20:439–444. https://doi.org/10.1080/08880010390220144.
3. Husain E, Whitehead S, Castell A, Thomas EE, Speert DP. 2005. Viridans streptococci bacteremia in children with malignancy: relevance of species identification and penicillin susceptibility. Pediatr Infect Dis J 24:563–566. https://doi.org/10.1097/01.inf.0000164708.21464.03.
4. García-de-la-Mària C, Pericas JM, Del Río A, Castañeda X, Vila-Farrés X, Armero Y, Espinal PA, Cervera C, Soy D, Falces C, Ninot S, Almela M, Mestres CA, Gatell JM, Vila J, Moreno A, Marco F, Miró JM. Hospital Clinic Experimental Endocarditis Study Group. 2013. Early *in vitro* and *in vivo* development of high-level daptomycin resistance is common in mitis group streptococci after exposure to daptomycin. Antimicrob Agents Chemother 57:2319–2325. https://doi.org/10.1128/AAC.01921-12.
5. Huang WT, Chang LY, Hsueh PR, Lu CY, Shao PL, Huang FY, Lee PI, Chen CM, Lee CY, Huang LM. 2007. Clinical features and complications of viridans streptococci bloodstream infection in pediatric hemato-oncology patients. J Microbiol Immunol Infect 40:349–354.
6. Shelburne SA, Sahasrabhojane P, Saldana M, Hui Y, Xiaoping S, Horstmann N, Thompson E, Flores AR. 2014. Streptococcus mitis strains causing severe clinical disease in cancer patients. Emerg Infect Dis 20:762–771. https://doi.org/10.3201/eid2005.130953.
7. Freifeld AG, Razonable RR. 2014. Viridans group streptococci in febrile neutropenic cancer patients: what should we fear? Clin Infect Dis 59:231–233. https://doi.org/10.1093/cid/ciu264.
8. Marron A, Carratala J, Gonzalez-Barca E, Fernandez-Sevilla A, Alcaide F, Gudiol F. 2000. Serious complications of bacteremia caused by viridans streptococci in neutropenic patients with cancer. Clin Infect Dis 31:1126–1130. https://doi.org/10.1086/317460.
9. Safdar A, Rolston KV. 2006. Vancomycin tolerance, a potential mechanism for refractory gram-positive bacteremia observational study in patients with cancer. Cancer 106:1815–1820. https://doi.org/10.1002/cncr.21801.
10. Prabhu RM, Piper KE, Baddour LM, Steckelberg JM, Wilson WR, Patel R. 2004. Antimicrobial susceptibility patterns among viridans group streptococci isolates from infective endocarditis patients from 1971–1986 and 1994 to–2002. Antimicrob Agents Chemother 48:4463–4465. https://doi.org/10.1128/AAC.48.11.4463-4465.2004.
11. Shelburne SA, Lasky RE, Sahasrabhojane P, Tarrand JT, Rolston KVI. 2014. Development and validation of a clinical model to predict the presence of β-lactam resistance in viridians group streptococci causing bacteremia in neutropenic cancer patients. Clin Infect Dis 59:223–230. https://doi.org/10.1093/cid/ciu260.
12. Ron-Bin H, Lin F-Y. 2006. Effect of penicillin resistance on presentation and outcome of non-enterococcal streptococcal infective endocarditis. Cardiology 105:234–239. https://doi.org/10.1159/000091821.
13. Sabella C, Murphy D, Drummond-Webb J. 2001. Endocarditis due to Streptococcus mitis with high-level resistance to penicillin and ceftriaxone. JAMA 285:2195. https://doi.org/10.1001/jama.285.17.2195.
14. Singh N, Poggensee L, Huang Y, Evans CT, Suda KJ, Bulman ZP. 2022. Antibiotic susceptibility patterns of viridans group streptococci isolates in the United States from 2010 to 2020. JAC Antimicrob Resist 19 4:dlac049. https://doi.org/10.1093/jacamr/dlac049.
15. Zapata B, Alvarez DN, Farah S, Garcia-de-la-Maria C, Miro JM, Sakoulas G, Bayer AS, Mishra NN. 2018. Prevention of high-level daptomycin-resistance emergence *in vitro* in *Streptococcus* mitis-oralis by using combination antimicrobial strategies. Curr Microbiol 75:1062–1067. https://doi.org/10.1007/s00284-018-1491-3.
16. Kebriaei R, Rice SA, Stamper KC, Seepersaud R, Garcia-de-la-Maria C, Mishra NN, Miro JM, Arias CA, Tran TT, Sullam PM, Bayer AS, Rybak MJ. 2019. Daptomycin dose-ranging evaluation with single-dose versus multidose ceftriaxone combinations against Streptococcus mitis/oralis in an ex vivo simulated endocarditis vegetation model. Antimicrob Agents Chemother 263:e00386-19. https://doi.org/10.1128/AAC.00386-19.
17. Yim J, Smith JR, Singh NB, Rice S, Stamper K, Garcia de la Maria C, Bayer AS, Mishra NN, Miró JM, Tran TT, Arias CA, Sullam P, Rybak MJ. 2017. Evaluation of daptomycin combinations with cephalosporins or gentamicin against *Streptococcus mitis* group strains in an in vitro model of simulated endocardial vegetations (SEVs). J Antimicrob Chemother 72:2290–2296. https://doi.org/10.1093/jac/dkx130.
18. Parrett A, Reed JM, Gardner SG, Mishra NN, Bayer AS, Powers R, Somerville GA. 2020. Metabolic changes associated with adaptive resistance to daptomycin in *Streptococcus mitis-oralis*. BMC Microbiol 20:162. https://doi.org/10.1186/s12866-020-01849-w.
19. Kebriaei R, Bayer AS, Lapitan CK, Rybak MJ, Somerville GA, Mishra NN. 2022. Activity of the lactate dehydrogenase inhibitor oxamic acid against the fermentative bacterium *Streptococcus mitis/oralis*: bactericidal effects and prevention of daptomycin resistance *in vitro* and in an *ex vivo* model. Antibiotics (Basel) 11:1409. https://doi.org/10.3390/antibiotics11101409.
20. Chambers HF, Basuino L, Hamilton SM, Choo EJ, Moise P. 2016. Daptomycin-β-lactam combinations in a rabbit model of daptomycin-nonsusceptible methicillin-resistant *Staphylococcus aureus* endocarditis. Antimicrobial Agents Chemother 60:3976–3979. https://doi.org/10.1128/AAC.00589-16.
21. Mishra NN, Tran TT, Arias CA, Seepersaud R, Sullam PM, Bayer AS. 2020. Strain-specific adaptations of Streptococcus mitis-oralis to Serial in vitro passage in daptomycin (DAP): genotypic and phenotypic characteristics. Antibiotics 9:520. https://doi.org/10.3390/antibiotics9080520.
22. Mishra NN, Tran TT, Seepersaud R, Garcia-de-la-Maria C, Faull K, Yoon A, Proctor R, Miro JM, Rybak MJ, Bayer A. 2017. Perturbations of phosphatidatecytidylyltransferase (CdsA) mediate daptomycin resistance in *Streptococcus mitis/oralis* by a novel mechanism. Antimicrob Agents Chemother 61:e02435-16. https://doi.org/10.1128/AAC.02435-16.
23. Garcia-de-la-Maria C, Xiong YQ, Pericas JM, Armero Y, Moreno A, Mishra NN, Rybak MJ, Tran TT, Arias CA, Sullam PM, Bayer AS, Miro JM. 2017. Impact of high-level daptomycin resistance in the *Streptococcus mitis* group on virulence and survivability during daptomycin treatment in experimental infective endocarditis. Antimicrob Agents Chemother 61:e02418-16. https://doi.org/10.1128/AAC.02418-16.
24. Pichardo C, Docobo-Pérez F, Pachón-Ibáñez ME, Jiménez-Mejías ME, García-Curiel A, Caballero-Granado FJ, Moreno-Maqueda I, Pachón J. 2005. Efficacy of β-lactams against experimental pneumococcal endocarditis caused by strains with different susceptibilities to penicillin. J Antimicrob Chemother 56:732–737. https://doi.org/10.1093/jac/dki304.