Impact of Dose-Administration Strategies of the Antistaphylococcal Lysin Exebacase, (EXE), in Addition to Daptomycin (DAP) in an Experimental Infective Endocarditis (IE) Model due to Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Abstract

Background: MRSA infections, especially involving the endovascular system (e.g., IE), are associated with unacceptably high morbidity and mortality rates. The use of bacteriophage-derived lysin, which acts as direct lytic agents, represents a novel adjunctive approach against virulent Gram-positive bacteria, such as MRSA. The current study examined the efficacy of DAP alone or DAP plus EXE administered on a single day using various dosing regimens, in a rabbit model of MRSA IE.

Method: Aortic valve IE due to MRSA strain MW2 was induced by the IV administration of ~1-2 x10^5 cfu in aortic-catheterized rabbits. At 24h post-infection, animals were randomized into one of 15 groups: **1)** controls; **2)** vehicle controls given once daily (**QD**); **3-15)** DAP alone (at 4 mg/kg iv **QD** x 4d; this dose yields significant but modest clearance of MRSA in experimental IE); DAP + EXE (given as an IV dose on the first day of DAP treatment only by 5-10 min slow bolus at (mg/kg): 0.70 **QD**, 0.35 **Q12h**, 0.23 **Q8h**, 0.35 **QD**, 0.175 **Q12h**, 0.117 **Q8h**, 0.09 **QD**, 0.045 **Q12h**, 0.03 **Q8h**, 0.06 **QD**, 0.03 **Q12h** or 0.03 **QD**. At 24h after the last DAP dose, three target organs were quantitatively cultured (cardiac vegetations; kidneys and spleen). Data for each organ were calculated as mean $\log_{10}$ cfu/g of tissue (± SD).

Results: Treatment with DAP alone caused ~2-3 $\log_{10}$ cfu/g reduction in MRSA densities in all three target tissues vs vehicle controls. All EXE doses given in addition to DAP, even at the lowest EXE dose (0.03 mg/kg), significantly reduced MRSA densities further in all target tissues vs DAP alone (~3 $\log_{10}$ cfu/g) and vehicle control groups (~6 $\log_{10}$ cfu/g). In general, DAP plus EXE given as a single dose trended towards better microbiologic efficacy than EXE given at Q12h or Q8h, although this difference was not statistically significant.

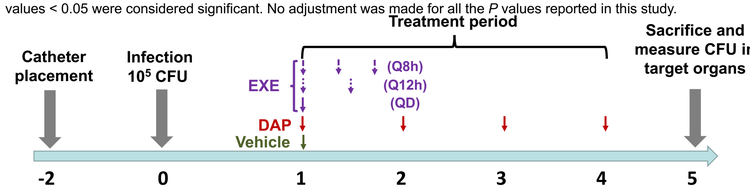
Conclusion: These results demonstrate that EXE, given at multiple dose strategies and at different dose-regimens, in addition to sublethal DAP, had significant efficacy in further decreasing MRSA densities in relevant target tissues in the IE model (vs DAP-alone and untreated controls). DAP plus a single dose of EXE trended to better efficacy than when it was administered in fractionated dose-strategies.

Methods

Strains. MRSA strain MW2 [CA-MRSA; USA400; MIC (µg/ml) – DAP (0.5)[1,2], EXE (1.0)[1,2]].

Experimental model of infective endocarditis (IE). A model of left-sided catheter-induced IE due to MRSA strain MW2 in rabbits[3] was used to examine the efficacy of EXE and DAP alone, and EXE in addition to DAP. Briefly, female New Zealand White rabbits (Harlan Laboratories; 2.3 to 2.5 kg body weight) underwent transcarotid-transaortic valve catheterization, and IE was induced by IV infection of ~1-2 x10^5 cfu of MW2 at 48h after catheterization. At 24h post-infection, animals were randomized into one of 15 groups (as showed below). On day 5, animals were sacrificed, and target tissues (cardic vegetations; kidney and spleen) were removed and quantitatively cultured. Tissue MRSA counts are given as the mean $\log_{10}$ CFU/g of tissue ± SD).

Statistical analysis. Two-tailed Student's *t* test was used to analyze the tissue MRSA counts between different groups. *P* values < 0.05 were considered significant. No adjustment was made for all the *P* values reported in this study.



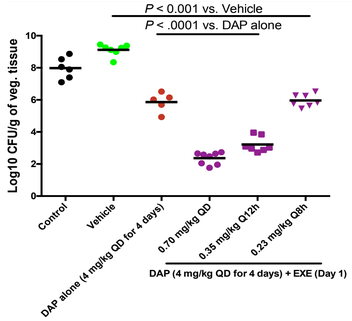
Treatment and control groups:

1. **Control (Non-treated and sacrificed at 24 hours post-infection)**
2. **Vehicle-treated (single IV on Day 1)**
3. **DAP alone (4 mg/kg, IV QD for 4 days)**
4. **EXE (QD, 0.03 mg/kg, IV on Day 1) + DAP**
5. **EXE (QD, 0.06 mg/kg, IV on Day 1) + DAP**
6. **EXE (QD, 0.09 mg/kg, IV on Day 1) + DAP**
7. **EXE (QD, 0.35 mg/kg, IV on Day 1) + DAP**
8. **EXE (QD, 0.7 mg/kg, IV on Day 1) + DAP**
9. **EXE (Q12h, 0.03 mg/kg, IV on Day 1) + DAP**
10. **EXE (Q12h, 0.045 mg/kg, IV on Day 1) + DAP**
11. **EXE (Q12h, 0.175 mg/kg, IV on Day 1) + DAP**
12. **EXE (Q12h, 0.35 mg/kg, IV on Day 1) + DAP**
13. **EXE (Q8h, 0.03 mg/kg, IV on Day 1) + DAP**
14. **EXE (Q8h, 0.117 mg/kg, IV on Day 1) + DAP**
15. **EXE (Q8h, 0.23 mg/kg, IV on Day 1) + DAP**

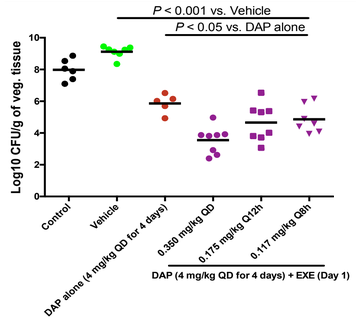
Results

Bacterial burden in cardiac (heart valve) vegetations following different EXE dosing strategies in addition to DAP

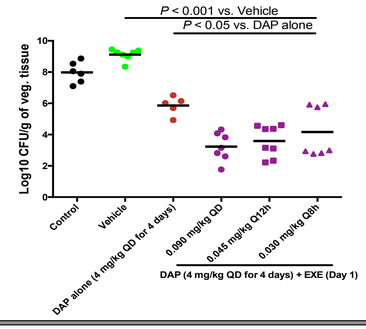
EXE dose fraction of 0.7 mg/kg + DAP



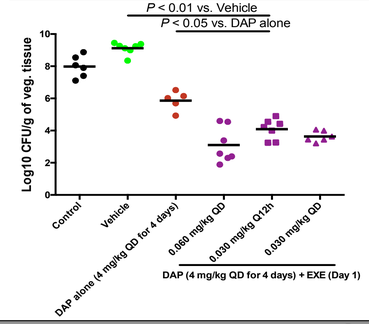
EXE dose fraction of 0.35 mg/kg + DAP



EXE dose fraction of 0.090 mg/kg + DAP



EXE dose fraction of 0.060 mg/kg + DAP



Mean (± SD) MRSA Densities in Other Tissues (Kidneys and Spleen) in the Rabbit IE Model.

Treatment	Dose Level (mg/kg)	Dose Frequency	Mean $\log_{10}$ CFU/g tissue ± SD	
			Kidneys	Spleen
Control	-	-	7.23 ± 0.93	6.98 ± 0.56
Vehicle	0	QD	8.23 ± 0.58	7.90 ± 0.48
DAP	4	QD	**4.62 ±1.16[a]**	**4.04 ± 0.87[a]**
EXE/DAP	0.7/4	SD/QD	**2.02 ± 0.45[a]**	**2.14 ± 0.51[a]**
EXE/DAP	0.35/4	Q12h/QD	**2.41 ± 0.45[a]**	**2.37 ± 0.64[a]**
EXE/DAP	0.23/4	Q8h/QD	**5.03 ± 0.14[a]**	**4.95 ± 0.18[a]**
EXE/DAP	0.35/4	SD/QD	**2.87 ± 0.42[a]**	**2.82 ± 0.58[a]**
EXE/DAP	0.175/4	Q12h/QD	**3.85 ± 0.51[a]**	**3.90 ± 0.33[a]**
EXE/DAP	0.117/4	Q8h/QD	**4.60 ± 0.50[a]**	**3.93 ± 0.31[a]**
EXE/DAP	0.09/4	SD/QD	**3.45 ± 0.55[a]**	**3.20 ± 0.74[a]**
EXE/DAP	0.045/4	Q12h/QD	**3.06 ± 0 20[a]**	**2.99 ± 0.55[a]**
EXE/DAP	0.03/4	Q8h/QD	**3.34 ± 0.94[a]**	**3.52 ± 0.80[a]**
EXE/DAP	0.06/4	SD/QD	**2.82 ± 0.40[a]**	**3.05 ± 0.42[a]**
EXE/DAP	0.03/4	Q12h/QD	**3.73 ± 0.33[a]**	**3.59 ± 0.38[a]**
EXE/DAP	0.03/4	SD/QD	**3.05 ± 0.22[a]**	**3.10 ± 0.43[a]**

Values in red fond indicate P < 0.05 vs. DAP alone;
[a] *P* < 0.01 vs. Vehicle

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Conclusion

Our findings demonstrated that EXE, given at multiple dose strategies and at different dose-regimens, in addition to sublethal DAP, had significant efficacy in further decreasing MRSA densities in target tissues in the IE model (vs DAP-alone and untreated controls). DAP plus a single dose of EXE trended to better efficacy than when it was administered in fractionated dose-strategies.

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