A-003

Assessment of Non-Antibiotic Therapeutic Agent Interactions on the Potency of Orally Administered Vancomycin

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ABSTRACT

Background: Antibiotic-associated diarrhea caused by *S. aureus* (SA) and *C. difficile* (CDIF) has become more frequent and serious in recent years, with oral vancomycin (VAN) and/or metronidazole as the preferred treatment. Since antibiotic treatment can occur while a patient is on other therapies, we designed experiments to assess the interaction of selected non-antibiotic therapeutic agents (NATA) with VAN.

Methods: Three SA strains with reproducible VAN MIC (1 µg/ml) were tested against 32 NATA compounds. Broth microdilution panels were prepared with $\log_2$ dilutions of a NATA, ranging from 10,000 to 156 or from 1,000 to 16 µg/ml with and without VAN at 1 µg/ml. The absence of growth when the NATA was tested alone indicated anti-SA activity while the presence of growth when the NATA was tested with VAN showed antagonism. Three CDIF strains were tested against a selected group of 13 NATA compounds using an agar dilution method.

Results: Antagonism for SA was only observed with acetaminophen, folic acid and lamivudine, all at very high concentrations (≥2,500 µg/ml). Four NATA demonstrated anti-SA activity at low levels (25 – 100 µg/ml), propanolol at 625 – 1,250 µg/ml and 7 additional compounds only at very high levels (5,000 – 10,000 µg/ml). No antagonism or inhibitory activity was observed with 18 NATA at the concentrations tested. Against CDIF strains no antagonism was observed when combined with VAN, however, 8 NATA showed anti-CDIF activity with MIC values ranging from 31 – 2,000 µg/ml.

NATA	Antagonism with SA	Anti-SA activity	Anti-CDIF activity
Acetaminophen	2,500 – 10,000 µg/ml	No	No
Folic acid	2,500 µg/ml	No	No
Lamivudine	5,000 – 10,000 µg/ml	No	No
Efavirenz, tegaserod	No	25 µg/ml	31 – 125 µg/ml
Methotrexate, amlodipine besylate	No	25 – 100 µg/ml	500/No µg/ml
Propanolol	No	625 – 1,250 µg/ml	1,000 – 2,000 µg/ml
Clonidine, gabapentin, FH[a]	No	5,000 – 10,000 µg/ml	No
LP, AA, AS[a]	No	5,000 – 10,000 µg/ml	125 – 2,000 µg/ml

a. FH=fexofenadine hydrochloride, LP=losartan potassium, AA=acetylsalicylic acid, AS=alendronate sodium.

Conclusions: VAN antagonism was observed with 3 NATAs against SA at extremely high levels and not for CDIF. The antibacterial activities of several NATA agents warrant further evaluation.

INTRODUCTION

Antibiotic-associated diarrhea caused by *Staphylococcus aureus* and *Clostridium difficile* has become more frequent and serious in recent years, with oral vancomycin and/or metronidazole as the preferred treatment. Since antibiotic treatment can occur while a patient is on other therapies, it is possible that non-antibiotic therapeutic agents could interfere with or even enhance the antibiotic activity.

The objective of the presented study was to screen for potential antagonistic interactions between vancomycin and other orally administered treatment compounds. We designed experiments to assess the interaction of selected non-antibiotic agents with vancomycin using a fixed concentration of vancomycin and serially diluted concentrations of each co-drug. We also evaluated the in vitro anti-*S. aureus* activity of each compound.

MATERIALS AND METHODS

Bacterial isolates:

- *Staphylococcus aureus* (3 strains) with reproducible vancomycin MIC results of 1 µg/ml.
- *Clostridium difficile* (3 strains) with vancomycin MIC values at 4 or 0.5 µg/ml.
  - REA type BI9, vancomycin MIC of 4 µg/ml
  - REA type Y1, vancomycin MIC of 0.5 µg/ml
  - REA type J9, vancomycin MIC of 0.5 µg/ml

Compounds tested in combination with vancomycin against *S. aureus*: Acetaminophen, Acetylsalicylic acid, Alendronate sodium, Amlodipine besylate, Atenolol, Clonidine, Cyclosporine, Efavirenz, Enalapril maleate, Estradiol, Fexofenadine hydrochloride, Folic acid, Gabapentin, Ibuprofen, Lamivudine, Levothyroxine, Lithium carbonate, Losartan potassium, Metformin hydrochloride, Methotrexate, Omeprazole, Potassium Iodine, Prednisone, Propanolol, Ranitidine hydrochloride, Ritonavir, Sildenafil citrate, Simvastatin, Tegaserod, Verapamil hydrochloride, Zalcitabine, and Zidovudine.

Compounds tested in combination with vancomycin against *C. difficile*: Alendronate sodium, Acetylsalicylic acid, Acetaminophen, Efavirenz, Fexofenadine hydrochloride, Folic acid, Gabapentin, Ibuprofen, Lamivudine, Losartan potassium, Methotrexate, and Tegaserod.

Susceptibility testing: Broth microdilution panels were prepared with two-fold dilutions of each non-antibiotic therapeutic agent, ranging from 10,000 to 156 µg/ml or from 1,000 to 16 µg/ml with and without vancomycin at 1 µg/ml. The absence of growth when the non-antibiotic therapeutic agent was tested alone indicated anti-*S. aureus* activity while the presence of growth when the non-antibiotic therapeutic agent was tested in combination with vancomycin showed antagonism.

Three *C. difficile* strains were tested against a selected group of 13 non-antibiotic therapeutic agents using an agar dilution method. Combination testing was performed using an agar dilution method according to Clinical and Laboratory Standards Institute (CLSI; M11-A7) recommendations. Each compound was tested over a range of eight concentrations beginning with 2,000 µg/ml (0.2% of full potency) except for folic acid, which started at 1,000 µg/ml (0.1%), using $\log_2$ dilutions (2,000; 1,000; 500; 250; 125; 63; 31 and 16 µg/ml or 1,000; 500; 250; 125; 63; 31; 16 and 8 µg/ml) with vancomycin added at a fixed concentration of 4 µg/ml for the *C. difficile* REA type BI9 strain or 0.5 µg/ml for the *C. difficile* REA type Y1 and *C. difficile* REA type J9 strains.

The pH of the stock solution containing the drug at the highest concentration in the dilution medium was measured and adjusted to physiologic levels (7.2-7.4), if necessary, by adding 1 N NaOH or 0.05 M HCl.

RESULTS

- Antagonism of the vancomycin activity against the *S. aureus* strains was only observed with acetaminophen, folic acid and lamivudine, all at very high concentrations (≥2,500 µg/ml; Table 1).
- Four non-antibiotic therapeutic agents (enfavirenz, tegaserod, methotrexate, and amlodipine), demonstrated anti-*S. aureus* activity at low levels (25 – 100 µg/ml), propanolol at 625 – 1,250 µg/ml and 6 additional compounds only at very high concentrations (5,000 – 10,000 µg/ml; Table 1).

Table 1. Evaluation of vancomycin antagonism and anti-*S. aureus* (SA) activity of selected non-antimicrobial compounds.

Compound/*S. aureus* strain	(MIC in µg/ml) Antagonism concentration	Anti-*S. aureus* activity
Atenolol, Cyclosporine, Enalapril maleate, Estradiol, Ibuprofen, Lithium carbonate, Levothyroxine sodium, Metformin hydrochloride, Omeprazole, Potassium iodine, Prednisone, Ranitidine hydrochloride, Ritonavir, Sildenafil citrate, Simvastatin, Verapamil hydrochloride, Zalcitabine and Zidovudine		
SA29213	No[a]	No
SA30-20C	No	No
SA117-26C	No	No
Acetaminophen		
SA29213	No	No
SA30-20C	10,000	No
SA117-26C	2,500	No
Acetylsalicylic acid		
SA29213	No	No
SA30-20C	No	No
SA117-26C	No	10,000
Alendronate sodium		
SA29213	No	10,000
SA30-20C	No	10,000
SA117-26C	No	10,000
Amlodipine besylate		
SA29213	No	63
SA30-20C	No	63
SA117-26C	No	63
Clonidine		
SA29213	No	5,000
SA30-20C	No	5,000
SA117-26C	No	5,000
Efavirenz		
SA29213	No	25
SA30-20C	No	25
SA117-26C	No	25
Fexofenadine hydrochloride		
SA29213	No	10,000
SA30-20C	No	10,000
SA117-26C	No	10,000
Folic acid		
SA29213	2,500	No
SA30-20C	2,500	No
SA117-26C	2,500	No
Gabapentin		
SA29213	No	5,000
SA30-20C	No	5,000
SA117-26C	No	10,000
Lamivudine		
SA29213	10,000	No
SA30-20C	5,000	No
SA117-26C	5,000	No
Losartan potassium		
SA29213	No	5,000
SA30-20C	No	5,000
SA117-26C	No	5,000
Methotrexate		
SA29213	No	25
SA30-20C	No	25
SA117-26C	No	100
Propanolol		
SA29213	No	625
SA30-20C	No	625
SA117-26C	No	1,250
Tegaserod		
SA29213	No	25
SA30-20C	No	25
SA117-26C	No	25

a. No – no antagonism interaction was detected or not inhibition at ≤10,000 µg/ml.

- No antagonism or anti-*S. aureus* inhibitory activity was observed for 18 non-antibiotic therapeutic agents at the concentrations tested.
- Against *C. difficile* strains, no antagonism was observed for any of the 13 non-antibiotic therapeutic agent compounds when combined with vancomycin.
- Nine non-antibiotic therapeutic agent compounds showed anti-*C. difficile* activity with MIC values ranging from ≤16 – 2,000 µg/ml; see Table 2.

Table 2. Evaluation of vancomycin antagonism and anti-*C. difficile* activity of selected non-antimicrobial compounds.

Compound/*C. difficile* strain	(MIC in µg/ml) Antagonism concentration	Anti-*C. difficile* activity
Acetaminophen, Fexofenadine hydrochloride, Folic acid, Gabapentin, and Lamivudine		
REA type BI9	No[a]	None
REA type Y1	No	None
REA type J9	No	None
Acetylsalicylic acid		
REA type BI9	No	1,000
REA type Y1	No	1,000
REA type J9	No	2,000
Alendronate sodium		
REA type BI9	No	125
REA type Y1	No	250
REA type J9	No	250
Efavirenz		
REA type BI9	No	125
REA type Y1	No	63
REA type J9	No	125
Ibuprofen		
REA type BI9	No	500
REA type Y1	No	500
REA type J9	No	500
Losartan potassium		
REA type BI9	No	1,000
REA type Y1	No	1,000
REA type J9	No	2,000
Methotrexate		
REA type BI9	No	500
REA type Y1	No	None
REA type J9	No	None
Propanolol		
REA type BI9	No	2,000
REA type Y1	No	1,000
REA type J9	No	2,000
Tegaserod		
REA type BI9	No	63
REA type Y1	No	31
REA type J9	No	63

a. No = no antagonism interaction was detected or no inhibition at ≤2,000 µg/ml.

CONCLUSIONS

- Vancomycin antagonism was observed for only three non-antibiotic therapeutic agents against *S. aureus* but only at extremely high concentrations.
- No antagonism of vancomycin activity was observed against *C. difficile* with any of the non-antibiotic therapeutic agents evaluated.
- The antibacterial activities of several non-antibiotic therapeutic agents against *S. aureus* (MIC, 25 to 10,000 µg/ml) or *C. difficile* (MIC, 31 to 2,000 µg/ml) strains warrant further evaluation.

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