In Vitro Antagonism with the Combination of Vancomycin and Clindamycin Against *Staphylococcus aureus*

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**KEY WORDS:** Vancomycin, clindamycin, drug interactions, antibiotics, staphylococcus

**ABSTRACT**

The activity of vancomycin in combination with clindamycin against methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) was evaluated. Fractional inhibitory concentration (FIC) testing and traditional time-kill experiments were performed, with each drug alone and in combination at concentrations ranging from 0.25-50x the minimum inhibitory concentration (MIC). Vancomycin and clindamycin MICs were 1, 0.25 and 1, 0.5 µg/mL, while FICs were 16 and 8 for the MRSA and MSSA strains respectively. Bacterial kill at 8 and 24 hours showed vancomycin 10, 20x-clindamycin 0.25-50x MIC to be highly antagonistic. Combinations of lower vancomycin concentrations (0.25-2x MIC) and high clindamycin concentrations (10-50x MIC) resulted in antagonism, whereas low vancomycin and clindamycin concentration combinations resulted in slightly better bacterial kill than either agent alone. Antagonism appears to be concentration dependent and varies among bacterial strains. Caution should be used if vancomycin and clindamycin are to be co-administered, especially in those lacking a functional immune system.

**INTRODUCTION**

Combination antibiotic therapy is routinely used in the discipline of infectious diseases. Reasons for combination therapy include: management of difficult to treat infections, treating polymicrobial infections, enhancing the spectrum of activity, reducing the emergence of resistance, and achieving synergy. However before combination therapy is initiated, clinicians must be aware of the nature of the interaction between the antibiotics to avoid undesirable pharmacodynamics. Clinicians must also consider the interaction among all antibiotics of a patient’s regimen, even when using two drugs targeted at different bacteria.
Both vancomycin and clindamycin have been available for over a decade, and there is a great deal of clinical experience using both drugs for the management of infections. Occasionally, clindamycin may be added to antibiotic regimens containing vancomycin with the goal of providing coverage against anaerobic bacteria. In this situation, both vancomycin and clindamycin have activity against Staphylococcus, raising the question of a potential interaction between these two agents. Previous data generated by Ho et al.\(^2\) suggested that the combination of vancomycin and clindamycin may be antagonistic in *Staphylococcus aureus* isolates. Such antagonism may have important clinical relevance, especially for a slowly bactericidal agent such as vancomycin, and could potentially result in therapeutic failure.

The primary purpose of this study was to evaluate a potential interaction between vancomycin and clindamycin in *Staphylococcus aureus* using clinical isolates of MRSA and MSSA.

**METHODS**

**Bacteria and Collection**

Two *Staphylococcus aureus* strains, one resistant to methicillin (MRSA, strain 402), and one sensitive to methicillin (MSSA, strain 065) were studied. The isolates were obtained from the blood of patients with bacteremia at Roswell Park Cancer Institute, Buffalo, NY.

**Antibiotics and In Vitro Susceptibility Testing**

Vancomycin (Sigma Aldrich, St. Louis, Mo), and clindamycin (Sigma Aldrich, St. Louis, Mo) minimum inhibitory concentrations (MICs) were determined in Mueller-Hinton broth (MHB; Difco Laboratories, Detroit, Mich) by the broth macrodilution method as described by the National Committee for Clinical Laboratory Standards.\(^3\) Stock antibiotic concentrations used for serial dilution and determination of MICs were verified by using the American Type Culture Collection (ATCC) control strain 29212 (*Enterococcus faecalis*). Minimum inhibitory concentrations for vancomycin and clindamycin were performed in triplicate on separate occasions yielding identical results.

**Time-kill Studies**

A series of static time-kill studies were performed comparing vancomycin and clindamycin alone versus combinations of each drug. Concentrations used for the series of experiments varied from sub to several multiples (0.25-20) of each organism’s vancomycin MIC, and 0.25-50x MIC for clindamycin. In all instances, the antibiotic concentrations used in the experiments were representative of clinically achievable plasma concentrations of the drug.\(^4,5,6\) Each experiment was conducted as follows. Bacteria were grown to the logarithmic phase by inoculating cation-supplemented MHB and incubating in a water bath at 37°C for 2 to 3 hours. One-milliliter samples consisting of approximately 10⁸ colony forming units (CFU) bacteria were added to 9 mL of MHB to result in an initial inoculum of 10⁹ CFU/mL. Samples (0.1mL) were collected at 0, 1, 3, 5, 8, and 24 hours to measure drug(s) effect on bacteria. Samples were serially diluted in cold normal saline at 4°C, and aliquots of 10 and 100 µL were plated in duplicate onto blood agar, and plates incubated for 24 hours at 35°C. Viable colonies, representative of a single bacterial cell, between 10 and 100 per plate were counted with a lower limit of detection of 10² CFU/mL. Bacteria colony counts (CFU/mL) were calculated and plotted against time to graphically assess drug effect of the various drugs alone and in combination.
Interaction Studies
Initial assessment of the interaction between vancomycin and clindamycin began by performing traditional checkerboard experiments for each drug combination. Methodology for preparing organism and drug stock was done in a similar manner as described above. Methodology for performing the checkerboard experiments was in accordance to those described previously. Fractional inhibitory concentration (FIC) indices of 0.5 was defined as synergy, whereas FIC indices of 1-4 or >4 defined as additivity or antagonism, respectively. Fractional inhibitory concentrations were determined after 24 hours of incubation at 35°C.

Further evaluation of the drug interaction was done by visually inspecting the bacterial time-kill curves, making note of the activity of each agent alone at various multiples of the MIC, and then comparing it to combination curves, looking for added or reduced activity at various time points. Synergism or antagonism was defined as either a 2-log increase or decrease in CFU/mL with the combination at 24 hours when compared to the most active single drug alone. Additivity was defined as less than 1-log change in activity at 24 hours. Bacterial killing at 8 and 24 hours was measured by subtracting the bacterial counts at 8 or 24 hours from the initial inoculum, with negative values indicative of net growth. The time point of 8 hours was included to aid in displaying drug effect for those instances where 24-hour re-growth had occurred.

RESULTS
Susceptibility Testing
Vancomycin and clindamycin MICs were 1, 0.25 µg/mL for the MRSA 402 strain, and 1, 0.5 µg/mL for the MSSA 065 strain, respectively. Vancomycin MICs were identical for both strains, while the MSSA strain demonstrated a slightly higher clindamycin MIC.

FIC Testing
Median FICs for the MRSA and MSSA strain were 16 and 8 respectively; indi-
cating the combinations of vancomycin and clindamycin to be antagonistic (FIC >4), and was consistently reproducible.

**Time-kill Studies**

As seen in Figure 1, vancomycin 0.25 and 0.5x MIC concentrations had very little activity, with the curves closely resembling growth control for both strains. Vancomycin 1-2x MIC essentially held the MRSA bacterial counts close to the initial inoculum; whereas in the MSSA strain 1x MIC allowed bacterial growth to occur throughout, and 2x MIC a 1.78 log₁₀ CFU/mL reduction in bacterial counts by 8 hours followed by regrowth. Vancomycin 10 and 20x MIC were both slowly active, with 10x MIC resulting in a 2.91 and 2.90 log₁₀ CFU/mL reduction and 20x MIC a 5.12 and 3.19 log₁₀ CFU/mL reduction at 24 hours, in the MRSA and MSSA strains respectively. Only vancomycin 20x MIC resulted in a bactericidal kill (ie ≥ 3 log₅₀) in both strains.

Figure 2 displays the time-kill curves of clindamycin 0.25-50x MIC alone.

Clindamycin 0.25-0.5x MIC was active, with clear separation from the growth control curve at all time points for both strains. Clindamycin 1 and 2x MIC essentially held bacterial counts close to initial inoculum in both strains, with 2x MIC resulting in a 1.92 log₁₀ CFU/mL reduction at 24 hours in the MRSA strain. Increasing clindamycin concentrations to 10, 20, and 50x MIC resulted in modest reductions in bacterial counts (0.96 log₁₀ CFU/mL maximal) in the MSSA strain. However clindamycin was highly active in the MRSA strain with 10, 20, and 50x MIC resulting in a 2.37, 2.38, and 4.30 log₁₀ CFU/mL reduction in bacterial counts at 24 hours. Only in the MRSA strain at 50x MIC was bactericidal activity noted.

Figure 3 shows time-kill curves for sub-MIC vancomycin concentrations of 0.5x MIC alone and in combination with 0.25-50x MIC clindamycin for both strains. Curves for the MRSA strain show that the addition of increasing clindamycin concentrations resulted in increased bacterial kill at 24 hours,

Figure 2. Time-kill studies for clindamycin alone versus methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA). GC; Growth control; C0.25, clindamycin 0.25x MIC; C0.5, clindamycin 0.5x MIC; C1, clindamycin 1x MIC; C2, clindamycin 2x MIC; C10, clindamycin 10x MIC; C20, clindamycin 20x MIC; C50, clindamycin 50x MIC. Log CFU/mL = Log₁₀ colony-forming units; MIC = minimum inhibitory concentration.
which plateaus at a maximum at 10x MIC. Curves for the MSSA strain reveal little effect of low clindamycin concentrations (0.25-2x MIC), however further increases in concentration to 10, 20, and 50x MIC resulted in additional bacterial kill at 24 hours. Using the MRSA strain as an example, comparing the vancomycin 0.5x/clindamycin 0.25-50x MIC combination curves to both drugs alone, a clear trend emerges. Since vancomycin 0.5x MIC alone had essentially no activity, the addition of any clindamycin resulted in increased bacterial kill at 24 hours. However, since clindamycin alone was active (Figure 2), making a comparison of the vancomycin-clindamycin combination relative to clindamycin demonstrates that aside from the 0.5/0.5x MIC combination, all other combinations show reduced bacterial kill at 24 hours. Although this trend clearly hints towards antagonism, the only combination that reached the definition of a ≥ 2-log10 reduction in bacterial kill at 24 hours compared to the most active single drug alone was the 0.5/50x MIC combination (clindamycin 50x MIC = 4.30 versus vancomycin-clindamycin 0.5/50x MIC = 1.90 log10 reduction at 24 hours). For the MSSA strain, 0.5/0.25-2x MIC combinations trended towards antagonism, however, 0.5/10-50x MIC combination resulted in slightly enhanced kill at 24 hours.

Time-kill curves representing concentrations of vancomycin at each organism’s MIC alone and with clindamycin are shown in Figure 4. For the MRSA strain, the addition of low concentrations of clindamycin (0.25-2x MIC) resulted in less activity than vancomycin 1x MIC alone. Further addition of clindamycin ranging from 10-50x MIC did result in additional bacterial kill at 24 hours compared to vancomycin 1x MIC alone. However, if a comparison is made between the clindamycin alone and vancomycin 1x MIC – clindamycin 10-50x MIC combination

Figure 4. Time-kill studies for vancomycin 1x MIC in combination with clindamycin versus methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA). GC, Growth control; V/C0.25, vancomycin 1x-clindamycin 0.25x MIC; V/C0.5, vancomycin 1x-clindamycin 0.5x MIC; V/C1, vancomycin 1x-clindamycin 1x MIC; V/C2, vancomycin 1x-clindamycin 2x MIC; V/C10, vancomycin 1x-clindamycin 10x MIC; V/C20, vancomycin 1x-clindamycin 20x MIC; V/C50, vancomycin 1x-clindamycin 50x MIC. Log CFU/mL = $\log_{10}$ colony-forming units; MIC = minimum inhibitory concentration.

Figure 5. Time-kill studies for vancomycin 20x MIC in combination with clindamycin versus methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA). GC, Growth control; V/C0.25, vancomycin 20x-clindamycin 0.25x MIC; V/C0.5, vancomycin 20x-clindamycin 0.5x MIC; V/C1, vancomycin 20x-clindamycin 1x MIC; V/C2, vancomycin 20x-clindamycin 2x MIC; V/C10, vancomycin 20x-clindamycin 10x MIC; V/C20, vancomycin 20x-clindamycin 20x MIC; V/C50, vancomycin 20x-clindamycin 50x MIC. Log CFU/mL = $\log_{10}$ colony-forming units; MIC = minimum inhibitory concentration.
curves, the activity of the combination is less than clindamycin alone, suggesting antagonism. This is clearly exemplified by comparing the 24 hour bacterial kill of 1/10, 20 and 50x MIC combinations (1.71, 1.71 and 2.28 log₁₀) to clindamycin 10, 20, and 50x MIC alone (2.33, 2.37 and 4.30 log₁₀) in the MRSA strain. Only the vancomycin 1x – clindamycin 50x MIC combination satisfied the criteria for antagonism (2.02 log₁₀ decrease in bacterial counts compared to clindamycin 50x alone). In contrast, combinations of clindamycin at any concentration resulted in slightly enhanced bacterial kill in the MSSA strain.

Curves representative of vancomycin 20x MIC alone and in combination with clindamycin 0.25-50x MIC are shown in Figure 5. Noting vancomycin 20x alone to be highly active (5.12 log₁₀ bacterial kill), it can be seen that the addition of any concentration of clindamycin to vancomycin at 20x MIC greatly reduced 24-hour bacterial kill in both strains. The magnitude of antagonism was most apparent in the MRSA strain, with all combinations resulting in a >2-log₁₀ reduction in 24 hours bacterial kill relative to vancomycin 20x alone (range 2.58-4.16 log₁₀). The same trend was apparent for the MSSA strain although not as pronounced, with 24 hour bacterial kill for 20/0.25-50x MIC combinations ranging from log₁₀ 1.34 to 1.88, less than vancomycin 20x MIC alone. Similar findings were observed for vancomycin-clindamycin 10/0.25-50x combinations, but not as pronounced.

Tables 1 and 2 display the calculated bacterial killing at 8 and 24 hours for all combination regimens tested in both strains. When comparing the bacterial kill...
Table 2. Bacterial Kill Measured Over Eight and Twenty-Four Hours Exposure in Strain Methicillin Sensitive Staphylococcus Aureus (065) *

<table>
<thead>
<tr>
<th>Clindamycin (x MIC)</th>
<th>BK$_{24}$ Clindamycin</th>
<th>BK$_{24}$ V0.25x</th>
<th>BK$_{24}$ V0.5x</th>
<th>BK$_{24}$ V1x</th>
<th>BK$_{24}$ V2x</th>
<th>BK$_{24}$ V10x</th>
<th>BK$_{24}$ V20x</th>
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<td>2.90</td>
<td>3.19</td>
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<td>-1.05</td>
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<td>2.04</td>
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<td>0.60</td>
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<td>1.51</td>
<td>2.65</td>
<td>0.02</td>
<td>0.97</td>
<td>1.82</td>
</tr>
</tbody>
</table>

* Dark grey shading denotes combination meets criteria for antagonism, light grey shading denotes combination had reduced activity compared to most active agent alone. BK indicates bacterial kill; and V, vancomycin.

kill for each drug alone in the MRSA and MSSA strains separately, it is apparent that both vancomycin and clindamycin were less active in the MSSA strain. Additionally, by looking at bacterial kill, a relationship emerges with respect to the nature and magnitude of the interaction and vancomycin-clindamycin concentration. With reference to bacterial kill at 24 hours in the MRSA strain, for instance, at vancomycin 20x MIC it appears that the addition of clindamycin at any concentration resulted in reduced bacterial kill. At vancomycin 10x MIC, a similar observation is made, but the amount of antagonism seen is not as intense compared to the vancomycin 20x series. For the vancomycin 0.25-2x MIC series, antagonism does not become apparent until clindamycin concentrations are at 2x MIC, after which, the bacterial kill appears to be progressively lessened (with respect to the more active agent, clindamycin) as clindamycin concentrations increase to 10, 20 and 50x MIC. This suggests not only that the vancomycin-clindamycin combination is antagonistic, but also the degree to which they are antagonistic is a concentration dependent phenomenon. The MSSA strain followed a similar trend with regards to the vancomycin 10 and 20x MIC combination series (highly antagonistic), but trended towards slightly enhanced bacterial kill at lower vancomycin-clindamycin concentrations.

**DISCUSSION**

Combination antimicrobial therapy continues to be an integral component in managing patients with difficult to treat
infections and polymicrobial infections. However, clinicians must be cognizant of the nature of the interaction between antibiotics used in patients, and care should be taken to avoid combining agents that may result in antagonism. As the number of possible antibiotic combinations that may be used clinically is large, many of these potential interactions have not been studied. The frequency of antagonistic combinations used clinically is unknown, but is likely under appreciated.

Vancomycin is a slowly bactericidal agent, eradicating bacteria much less rapidly than other drugs, such as oxacillin. Therefore, the addition of any agent that further decreases the activity of vancomycin is of significant concern. The results of these studies provide evidence of antagonism between vancomycin and clindamycin, and suggest that these agents should not be co-administered. Antagonism was observed with both FIC testing and time-kill curves, thereby validating the conclusions of each method of interaction assessment. Furthermore, our results concur with the previous findings of Ho et al., which demonstrated combinations of vancomycin-clindamycin or vancomycin-oxacillin to be either indifferent or antagonistic in approximately 50% of Staphylococcus aureus strains tested. A detailed mechanistic explanation as to why the combination of vancomycin and clindamycin are antagonistic is unknown. It is possible that a similar scenario is occurring as with co-administration of β-lactams and bacteriostatic agents where antagonism is frequently observed. As clindamycin is bacteriostatic and vancomycin is bactericidal, the resulting reduction in bacterial growth from the protein synthesis inhibitor (clindamycin) may blunt the effect the cell wall active agent (vancomycin) that works primarily on actively growing cells.

Our results highlight the interesting concept of interaction concentration dependence, which should be considered in antimicrobial interaction analyses. As seen in the bacterial time-kill plots (Figures 3-5), the addition of clindamycin to vancomycin at high concentrations consistently reduced the bacterial kill of vancomycin. Combinations at lower vancomycin concentrations (0.25–2x MIC) trended towards reduced bacterial kill, but frank antagonism was not reached until high concentrations of clindamycin were achieved. Although the concept of antimicrobial interactions being concentration dependent may seem intuitively apparent, it has not been rigorously described in the literature. It appears that either enhanced or reduced antimicrobial activity may result when the concentration of drug A is low and B high or vice versa. This may have an impact on the appropriate timing and sequence of administration of the drugs due to intrinsic differences in pharmacokinetics. Alternatively, sequence of administration may be the most important factor in the nature of the interaction. An example of this has been reported using an in vitro mycotic infection model simulating the human pharmacokinetics of combinations of fluconazole and amphotericin B. It was found that fluconazole administered 8 hours prior to amphotericin B reduced the fungicidal activity of amphotericin B to fungistatic activity similar to that seen with administration of fluconazole alone. Similarly, antagonism has been demonstrated in a dog model of pneumococcal meningitis only when chloramphenicol was administered prior to penicillin.

It appears that differences in the nature and magnitude of interaction between vancomycin and clindamycin vary with bacterial strains. This was seen with our MSSA strain showing less antagonism than the MRSA strain at
high vancomycin-clindamycin concentrations, and even slightly enhanced activity at low vancomycin-clindamycin concentrations. Comparing MIC values, the vancomycin MIC was 1 μg/mL in both strains while the clindamycin MIC was slightly higher in the MSSA strain. Clearly by looking at the time-kill curves and bacterial kill for both drugs alone they were much less active in the MSSA strain. It therefore appears that the blunting of antagonism observed in the MSSA strain is a consequence of the two drugs being individually less active, and that the magnitude of antagonism is, at least in part, a function of antimicrobial potency. Although both staphylococcus strains tested consistently demonstrated antagonism between vancomycin and clindamycin, additional strains of MRSA and MSSA should be tested before one should conclude that the combination is definitively antagonistic in all Staphylococcus aureus.

There are several reasons as to why metronidazole is preferred over clindamycin for anaerobic coverage. These include the greater incidence of adverse effects and associated increased risk of Clostridium difficile observed with clindamycin.\textsuperscript{10,11} Our finding of antagonism with vancomycin and clindamycin provides an additional reason as to why clinicians should avoid co-administration of these two antimicrobials, and favors the use of metronidazole plus vancomycin over the combination of clindamycin and vancomycin.

Traditional time-kill methodology to assess antibiotic drug interactions offers numerous advantages over the fractional inhibitory concentration (checkerboard) method. Not only is bacterial quantification made, but is accomplished serially over time. This allows for an assessment of the time course of drug effect on bacteria, thereby, not relying simply on the presence or absence of visible bacterial growth after a predefined time interval.

However, even the traditional time-kill method has some limitations. Only static, non-fluctuating drug concentrations are being used to study the interaction. It is therefore not entirely representative of the in vivo situation, where drug concentrations change over time. In vitro pharmacokinetic models attempt to account for this limitation by simulating drug pharmacokinetics.\textsuperscript{12} However, as with time-kill studies, all in vitro infection models are confounded by factors including growth media, growth conditions, lack of host immune system, error in antibiotic concentration, inoculum size, bacterial adherence, use of appropriate bacterial endpoints for interaction assessment, protein binding and even fundamental assumptions pertaining to interaction analysis of the data.\textsuperscript{13} All of these factors will continue to be problematic regardless of whether traditional time-kill methods or more sophisticated in vitro pharmacodynamic models are utilized. However, one cannot ignore the fact that data generated from time-kill studies demonstrated that combinations of β-lactams and aminoglycosides are synergistic; an effect that has since been validated in the treatment of human infections.\textsuperscript{14,15} Another example of validation of time-kill interaction results is antagonism seen with the use of tetracycline antibiotics with β-lactams; an interaction that has since been shown to be clinically significant.\textsuperscript{16,17}

**CONCLUSION**
Combining vancomycin with clindamycin against Staphylococcus aureus resulted in significant antagonism. The degree of antagonism appears to be a concentration dependent process, and bacterial strain differences are noted. Clinicians should avoid co-administration of vancomycin and clindamycin when treating serious infections or infections in immunocompromised hosts in
order to avoid potential in vivo antagonism and possible therapeutic failure.

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