J. Pharm. Pharmacol. 1999, 51: 1333–1337
Received April 7, 1999
Accepted June 17, 1999

The Antimicrobial Activity of Vancomycin in the Presence and Absence of Sodium Carboxymethyl Starch

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Abstract

The purpose of this work was to determine any effects the presence of sodium carboxymethyl starch may have on the antimicrobial activity of vancomycin given a previously described interaction between vancomycin and sodium carboxymethyl starch. In particular, the in-vitro activity of vancomycin against two clinically relevant bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, was studied in the presence of varying concentrations of sodium carboxymethyl starch.

From two independent studies conducted using an agar dilution method, it appeared that the binding of vancomycin to sodium carboxymethyl starch had no effect on the in-vitro antimicrobial activity of vancomycin. The minimum inhibitory concentration of vancomycin against *S. aureus* in the presence of as much as $1\,mg\,mL^{-1}$ sodium carboxymethyl starch was similar to that of the control where no sodium carboxymethyl starch was added ($1–4\,\mu g\,mL^{-1}$ vs $1–2\,\mu g\,mL^{-1}$, respectively). Likewise, the minimum inhibitory concentration of vancomycin against *E. faecalis* in the presence of $1\,mg\,mL^{-1}$ sodium carboxymethyl starch was also similar to that of the control where no sodium carboxymethyl starch was added ($1–4\,\mu g\,mL^{-1}$ vs $1–4\,\mu g\,mL^{-1}$, respectively). However, there may be factors in the in-vitro method, such as high ionic strength, that could disrupt the interaction between vancomycin and sodium carboxymethyl starch. Therefore, the possibility of diminished vancomycin activity in-vivo cannot be ruled out. A small percentage (8–10%) of vancomycin was determined to be bound to sodium carboxymethyl starch in broth media.

Given these results, the impact of sodium carboxymethyl starch on the in-vitro antimicrobial activity of vancomycin is expected to be minimal. Binding studies could not be conducted with gelled agar due to its semi-solid state.

Antimicrobial activity is the key performance criterion for screening antibiotic compounds. Determination of in-vitro activity is the most powerful tool for estimating the effectiveness of antibiotics against different bacterial strains and, hence, their potential for use in treating bacterial infections.

Numerous researchers have determined that antibiotic activity can be reduced due to interactions with excipients or trace metal contaminants. One research group demonstrated that streptomycin activity could be enhanced by an interaction with sodium alginate (El-Shibini et al 1971). Sodium alginate has historically been used as a tablet binder. The interaction was electrostatic in nature, resulting from the attraction of the basic amine groups on streptomycin to the acidic uronic groups on sodium alginate. The prolonged activity of the streptomycin–alginate complex was demonstrated in rabbits using streptomycin sulphate as a comparator. Spectrophotometric shifts in absorption were used to determine the interaction of norfloxacin with metal ions such as aluminium, magnesium, and calcium (Alkaysi et al 1992). Microbiological studies indicated decreased activity of norfloxacin in the presence of these metal ions. The antimicrobial activity of hexylresorcinol against Gram-negative and Gram-positive bacteria was demonstrated to be reduced in the presence of polyvinylpyrrolidone, a polymeric material commonly used as a tablet binder (Polli & Frost 1969).

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Sodium carboxymethyl starch (sodium starch glycolate) is a potent disintegrant used in various solid oral dosage forms. The use of sodium carboxymethyl starch as a disintegrant in a solid dosage form containing vancomycin could potentially result in alteration of vancomycin antimicrobial activity due to an interaction described previously (Claudius & Neau 1996). This possible interaction requires the effects of sodium carboxymethyl starch on the activity of vancomycin to be investigated through controlled studies. Using various bacterial strains, we have compared the minimum inhibitory concentrations of vancomycin alone and in the presence of sodium carboxymethyl starch to reveal any potential effects the interaction may present. Where possible, binding studies of vancomycin and sodium carboxymethyl starch in appropriate microbiological test media could aid in predicting potential effects on in-vitro activity.

Materials and Methods

Materials

Vancomycin was obtained as the hydrochloride salt form from ICN Biochemicals (Los Angeles, CA) and was used without further purification. The labelled microbiological potency of the vancomycin lot (No. 75262) used was $1084\,\mu g\,mg^{-1}$. Sodium carboxymethyl starch was available as Explotab from Edward Mendell Co. (Patterson, NY). Dehydrated Mueller Hinton agar was obtained from Remel (Lenexa, KS). Dehydrated Mueller Hinton and Mueller Hinton II broths were obtained from Becton Dickinson (Cockeysville, MD). Purified water, USP, was used for the preparation of agar and broth media. Sterile Water for Injection was used to prepare all vancomycin stock solutions and subsequent dilutions.

Bacterial control strains (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) were obtained from Remel (Lenexa, KS). All test strains were clinical isolates from Hoechst Marion Roussel, Inc. antibiotic efficacy studies.

Sterile pipettes and test tubes were used with all samples for processing steps following the autoclaving of agar. Aseptic handling techniques were used to minimize extraneous microbial contamination.

Methods

Agar methods are widely used for microbiological assay and are recognized as established techniques in the pharmaceutical literature (Tarcza & Garth 1978). An agar dilution method, as described by the National Committee for Clinical Laboratory Standards (NCCLS 1997), was used to determine the antimicrobial susceptibility of *S. aureus* and *E. faecalis*.

Agar media were prepared according to the manufacturer's recommended procedures and the NCCLS guidelines. The effect of the concentration of sodium carboxymethyl starch was studied at 0, 1, 10, $100\,\mu g\,mL^{-1}$ in study 1, and at 0, 100, and $1000\,\mu g\,mL^{-1}$ in study 2. Sodium carboxymethyl starch was added to the dehydrated agar before the addition of water. This powder mixture was dispersed in water and boiled for 1 min until the agar was fully dissolved. All agar was heat sterilized at 121°C for 10 min in 25-mL glass test tubes. The agar was allowed to cool to a constant temperature of $50° \pm 2°C$ and was thereafter maintained in a constant temperature water bath.

All vancomycin solutions were prepared by serial dilution of a $320\,\mu g\,mL^{-1}$ stock solution, resulting in 10 dilution samples. The final concentration of vancomycin used in the test procedure ranged from 0·0625 to $32\,\mu g\,mL^{-1}$.

Two millilitres of each vancomycin dilution sample was added to approximately 18 mL molten agar (nominally $50° \pm 2°C$) in test tubes. Each mixture was vortexed to ensure uniformity and then poured onto plastic Petri dishes on a level table to a depth of 3–4 mm. The contents of each Petri dish were allowed to solidify at room temperature, and were used the same day.

During the solidification of each agar sample, a bacterial inoculum from each of the 12 clinical isolates and two control strains of *S. aureus* and *E. faecalis* was prepared. The concentration of bacteria in each inoculum was standardized by measuring optical density at 530 nm on a variable wavelength spectrophotometer (Spectronic 20, Milton Roy, Rochester, NY). The optical density standard of the inoculum corresponded to a level of turbidity equal to 0·5 on the McFarland nephelometric scale (McFarland 1907) which resulted in each inoculum having a concentration of approximately $1–2 \times 10^8$ colony forming units mL^{-1}. Dilution of the inoculum was made with sterile broth where necessary.

Each agar plate was inoculated by placing 1–$2\,\mu L$ inoculum on the agar surface using an inoculum replicating device. A growth control plate with no vancomycin present was inoculated first, followed by agar plates from the lowest to highest vancomycin concentration. After a complete set of plates had been inoculated, the replicating device was placed on a second growth control plate with no vancomycin present to determine carry-over of

vancomycin during the subsequent inoculation. In addition, one agar plate with no vancomycin or sodium carboxymethyl starch was prepared as a control for extraneous growth. The small amount of liquid present from the inoculum was allowed to evaporate in a laminar flow hood (approx. 10 min). All agar plates were then inverted and incubated at 35°C (Lab-Line Environette, Lab-Line Instruments, Melrose, IL) under a normal atmosphere for approximately 18 h.

Each agar plate was visually examined for growth the following day using a light-assisted magnifying reader against a black background. The minimum inhibitory concentration was determined by the lowest concentration of vancomycin that completely inhibited growth, defined as three or less colonies present per inoculum.

Mueller Hinton and Mueller Hinton II broths were prepared for HPLC binding studies according to the manufacturer's recommended procedures for use in standard microbiological assays. Broth samples were prepared with vancomycin ($0{\cdot}5\,mg\,mL^{-1}$) alone and with sodium carboxymethyl starch present ($1\,mg\,mL^{-1}$). Sample blanks were run to verify that the components in the broth did not interfere with the isolation and quantification of vancomycin.

Results and Discussion

Inspection of the growth control plates indicated no carry-over of vancomycin during the inoculation process. In addition, there was no microbial growth observed in the negative control plate.

The acceptable quality control minimum inhibitory concentration range for vancomycin in the presence of *S. aureus* (ATCC 29213) and *E. faecalis* (ATCC 29212), is $0{\cdot}5–2\,\mu g\,mL^{-1}$ and $1–4\,\mu g\,mL^{-1}$, respectively, according to NCCLS guidelines. The minimum inhibitory concentrations for vancomycin against *S. aureus* and *E. faecalis* ATCC organisms were determined to be $1\,\mu g\,mL^{-1}$ and $4\,\mu g\,mL^{-1}$, respectively. The ATCC control strains of *S. aureus* and *E. faecalis* demonstrated appropriate susceptibility to vancomycin in the standard NCCLS agar, indicating suitable quality control.

The minimum inhibitory concentration of vancomycin against *S. aureus* in the presence of sodium carboxymethyl starch is shown in Table 1. From Table 1, the minimum inhibitory concentration of vancomycin against some *S. aureus* strains tends to increase slightly with increasing sodium carboxymethyl starch concentration. Specifically, staphylococcal strains SA1582, SA1866, and SA1902 exhibited slightly decreased susceptibility to vancomycin in the presence of sodium carboxymethyl starch. The effect of sodium carboxymethyl starch on the minimum inhibitory concentration of vancomycin against *E. faecalis* strains is shown in Table 2. The growth of strains EF1017, EF1022, EF1084, and EF1484 was less inhibited by vancomycin with increasing sodium carboxymethyl starch concentration.

A hypothesis was developed based on these findings that the binding of vancomycin to sodium carboxymethyl starch was reducing the free concentration of vancomycin in the agar and thus, a

Table 1. The effect of sodium carboxymethyl starch (NaCMS) concentration on the minimum inhibitory concentration of vancomycin against strains of *Staphylococcus aureus*. Study 1 data.

Strain	Minimum inhibitory concentration ($\mu g\,mL^{-1}$)			
	No NaCMS	NaCMS $1\,\mu g\,mL^{-1}$	NaCMS $10\,\mu g\,mL^{-1}$	NaCMS $100\,\mu g\,mL^{-1}$
SA1496	1·0	1·0	1·0	1·0
SA1537	1·0	1·0	1·0	1·0
SA1561	2·0	2·0	2·0	2·0
SA1582	1·0	1·0	1·0	2·0
SA1856	1·0	2·0	1·0	2·0
SA1866	1·0	1·0	2·0	4·0
SA1902	1·0	2·0	2·0	2·0
SA1909	1·0	1·0	1·0	1·0
SA2106	1·0	1·0	1·0	1·0
SA3467	1·0	2·0	1·0	2·0
SA3484	1·0	1·0	1·0	1·0
SA3789	2·0	2·0	2·0	2·0
SA29213 (Control)	1·0	1·0	1·0	1·0

Table 2. The effect of sodium carboxymethyl starch (NaCMS) concentration on the minimum inhibitory concentration of vancomycin against strains of *Enterococcus faecalis*. Study 1 data.

Strain	Minimum inhibitory concentration ($\mu g\,mL^{-1}$)			
	No NaCMS	NaCMS $1\,\mu g\,mL^{-1}$	NaCMS $10\,\mu g\,mL^{-1}$	NaCMS $100\,\mu g\,mL^{-1}$
EF1017	2·0	4·0	4·0	4·0
EF1022	2·0	4·0	4·0	4·0
EF1084	1·0	1·0	1·0	2·0
EF1100	1·0	1·0	1·0	1·0
EF1178	4·0	4·0	4·0	4·0
EF1262	4·0	4·0	4·0	4·0
EF1484	1·0	1·0	2·0	2·0
EF1543	1·0	1·0	1·0	1·0
EF1563	4·0	4·0	4·0	4·0
EF1568	4·0	4·0	4·0	4·0
EF1587	4·0	4·0	4·0	4·0
EF1595	8·0	8·0	8·0	8·0
EF29212 (Control)	4·0	4·0	4·0	4·0

higher total concentration of vancomycin was needed to achieve the same microbiological endpoint. It should be noted, however, that the minimum inhibitory concentration test employed in these studies could not statistically distinguish between a twofold difference in the minimum inhibitory concentration level.

Considering the small effects observed in the preliminary study, a second study using broader sodium carboxymethyl starch concentration ranges was conducted. The same ATCC control strains were run to demonstrate adequate quality control. Minimum inhibitory concentration determinations for these control strains were identical to those observed in study 1, $1\,\mu g\,mL^{-1}$ and $4{\cdot}0\,\mu g\,mL^{-1}$ for *S. aureus* and *E. faecalis* organisms, respectively. As in the preliminary study, the growth control plates indicated proper performance of the methods and procedures used in the study.

The effect of sodium carboxymethyl starch concentration on the minimum inhibitory concentration of vancomycin against *S. aureus* strains is reported in Table 3. The results from study 2 clearly do not support the findings from study 1. The presence of sodium carboxymethyl starch appears to have no effect on the in-vitro activity of vancomycin against the *S. aureus* strains studied. The effect of sodium carboxymethyl starch on the minimum inhibitory concentration of vancomycin against *E. faecalis* strains is shown in Table 4. Again, results from study 2 do not support the findings from study 1. The strains that were shown to have slightly higher minimum inhibitory concentrations with increasing sodium carboxymethyl starch concentration in the first study are not affected by the presence of as much as $1\,mg\,mL^{-1}$ sodium carboxymethyl starch.

Together, these two studies suggest that the presence of sodium carboxymethyl starch does not influence the in-vitro antimicrobial activity of vancomycin. However, there may be factors (e.g. ionic strength) that are inherent to the in-vitro method that may disrupt the binding interaction reported earlier. As described previously, the interaction of glycopeptide antibiotics and sodium carboxymethyl starch is best represented by an electrostatic attraction mechanism. Characteristically, electrostatic attraction mechanisms are strongly dependent upon pH and ionic strength. The interaction between vancomycin and sodium carboxymethyl starch is most pronounced at neutral pH and low ionic strength ($< 20\,mM$) where the interaction is favoured by the ionized forms of vancomycin and sodium carboxymethyl starch. The likelihood of in-vivo ionic strength being less than 20 mM is probably small, and this indicates reduced opportunities for a vancomycin–sodium carboxymethyl starch interaction in-vivo.

The pH of the agar in the NCCLS method is $7{\cdot}3 \pm 0{\cdot}1$. At this pH, the adsorption of vancomycin onto sodium carboxymethyl starch is favoured. However, the ionic strength of the agar medium is estimated to be greater than 20 mM, which would again decrease the likelihood of the interaction of vancomycin and sodium carboxymethyl starch. The estimation of the ionic strength of the agar is based on the fact that the manufacturers of Mueller Hinton agar (Remel and Becton Dickinson) routinely

Table 3. The effect of sodium carboxymethyl starch (NaCMS) concentration on the minimum inhibitory concentration of vancomycin against *Staphylococcus aureus* strains. Study 2 data.

Strain	Minimum inhibitory concentration ($\mu g\,mL^{-1}$)		
	No NaCMS	NaCMS $100\,\mu g\,mL^{-1}$	NaCMS $1000\,\mu g\,mL^{-1}$
SA1496	1·0	1·0	1·0
SA1537	1·0	1·0	1·0
SA1561	1·0	1·0	1·0
SA1582	2·0	1·0	2·0
SA1856	2·0	1·0	1·0
SA1866	1·0	1·0	1·0
SA1902	2·0	1·0	2·0
SA1909	1·0	1·0	1·0
SA2106	2·0	1·0	4·0
SA3467	2·0	1·0	1·0
SA3484	1·0	1·0	1·0
SA3789	2·0	2·0	2·0
SA29213 (Control)	1·0	1·0	1·0

Table 4. The effect of sodium carboxymethyl starch (NaCMS) concentration on the minimum inhibitory concentration of vancomycin against *Enterococcus faecalis* strains. Study 2 data.

Strain	Minimum inhibitory concentration ($\mu g\,mL^{-1}$)		
	No NaCMS	NaCMS $100\,\mu g\,mL^{-1}$	NaCMS $1000\,\mu g\,mL^{-1}$
EF1017	4·0	4·0	4·0
EF1022	4·0	4·0	4·0
EF1084	1·0	1·0	1·0
EF1100	1·0	1·0	1·0
EF1178	4·0	4·0	4·0
EF1262	4·0	4·0	4·0
EF1484	1·0	1·0	1·0
EF1543	1·0	1·0	1·0
EF1563	4·0	4·0	4·0
EF1568	4·0	4·0	4·0
EF1587	4·0	4·0	4·0
EF1595	4·0	4·0	4·0
EF29212 (control)	4·0	4·0	4·0

supplement their agar and broth products with magnesium and calcium ions to adjust the performance of these media with a standard set of quality control organisms.

Divalent ions, such as magnesium and calcium, strongly contribute to the ionic strength of the medium with a factor four-times their molar concentrations. Therefore, the presence of supplemented cations and other ionic species (e.g. protein, modified carbohydrates) in Mueller Hinton agar would contribute to the ionic strength and, thus, may discourage the interaction of glycopeptide antibiotics and sodium carboxymethyl starch. Finally, the gelled agar might reduce the mobility of vancomycin and not allow the intimate contact necessary for vancomycin molecules to interact with the insoluble sodium carboxymethyl starch substrate.

Since the probability of the interaction occurring is diminished with the reduced mobility of vancomycin molecules in the gelled agar, binding studies were conducted using HPLC in Mueller Hinton and Mueller Hinton II broths. The composition of Mueller Hinton and Mueller Hinton II broths is identical except for the higher concentrations of magnesium and calcium ions in the Mueller Hinton II broth. In addition, the composition of Mueller Hinton broth and Mueller Hinton agar is similar, except for the presence of agar. Results from binding studies indicated that approximately 8–10% vancomycin is bound to sodium carboxymethyl starch in both Mueller Hinton and Mueller Hinton II broths. Under these conditions, these results suggest that the effect of sodium carboxymethyl starch on the minimum inhibitory concentration of vancomycin would be minimal and undetectable given such a low level of bound vancomycin present in the microbiological test media.

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