Prolonged bacterial exposure to minocycline/rifampicin-impregnated vascular catheters does not affect antimicrobial activity of catheters

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Objectives: We assessed the in vitro effect of exposing various bacteria to minocycline/rifampicin-impregnated vascular catheters on the antimicrobial activity of the catheters and the antimicrobial susceptibility of tested organisms.

Methods: Segments of minocycline/rifampicin-impregnated catheters were placed in agar plates inoculated with methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant S. aureus (MRSA), methicillin-resistant Staphylococcus epidermidis (MRSE) and vancomycin-resistant Enterococcus (VRE). Zones of inhibition were measured at 24 h, and colonies from the edge of this zone were retrieved after 72 h and inoculated onto new agar plates. A total of seven 72 h cycles were completed. We then measured the MICs of minocycline, rifampicin, vancomycin and linezolid for the collected strains.

Results: The zones of inhibition of the four organisms remained stable after 21 days of sequential exposure to the impregnated catheters. The MICs of the antimicrobials remained constant, except for the MICs of rifampicin for MRSA and linezolid for MRSE, which increased slightly but remained within the susceptible range.

Conclusions: Minocycline/rifampicin-impregnated catheters remain effective against MSSA, MRSA, MRSE and VRE, as evidenced by stable zones of inhibition following 21 day sequential exposure to these catheters. The increase in MIC of rifampicin for MRSA may be clinically relevant if the catheter remains in place for >12 days though the strain remained susceptible to minocycline, there was no concurrent increase in the MIC of other tested drugs, and the zones of inhibition remained stable.

Keywords: minocycline/rifampicin-impregnated catheters, MIC, antimicrobial resistance

Introduction

Indwelling vascular catheters are the most common cause of nosocomial bloodstream infections, particularly among critically ill patients, haemodialysis-dependent subjects and persons receiving chemotherapy. Catheter-related bloodstream infections have substantial impact on morbidity, mortality, duration of hospital stay and overall cost of medical care. Preventive strategies that clinically protect against catheter-associated bacteraemia include the use of mechanical barrier precautions during insertion of the central venous catheter (CVC), skin antisepsis with chlorhexidine and impregnating CVCs with antibiotics or antisepsics.1 Prospective, randomized, multicentre clinical trials have demonstrated that minocycline/rifampicin-impregnated CVCs significantly prevent bloodstream infections when compared to un-impregnated catheters2,3 and are associated with a 12-fold lower rate of infection than catheters impregnated with chlorhexidine and silver sulfadiazine.3 Minocycline/rifampicin-impregnated catheters also decrease the length of stay and mortality in critically ill persons4 and haemodialysis patients.5 Use of these anti-infective catheters was found to be economically beneficial in decision model analyses with potential savings of approximately $10 000 for each prevented case of catheter-related bloodstream infection.6

Although there has been some concern that widespread use of antimicrobial-impregnated catheters might be associated with the emergence of drug resistance, this phenomenon has not been clinically noted. The objective of this in vitro study is to assess
the impact of exposing various Gram-positive bacteria to minocycline/rifampicin-impregnated vascular catheters on the antimicrobial activity of these catheters and the antimicrobial susceptibility of tested organisms.

Materials and methods

Organisms

We obtained one strain each of methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant S. aureus (MRSA), methicillin-resistant Staphylococcus epidermidis (MRSE) and vancomycin-resistant Enterococcus (VRE) from patients with catheter-associated bacteremia. The organisms were frozen in trypticase soy broth with 10% glycerol at −80°C until used.

Catheters

Triple-lumen, 7-French, minocycline/rifampicin-impregnated catheters were obtained from Cook Critical Care Inc. (Bloomington, IN, USA). Previous examination by HPLC showed that these catheters contain 11.08 mg of minocycline and 10.50 mg of rifampicin per 20 cm of catheter.

Exposure of bacteria to impregnated catheters

These experiments were done in triplicate. The inoculum was prepared by placing grown colonies of bacteria in normal saline and adjusting the turbidity of the bacterial suspension by a spectrophotometer to 0.5 McFarland units. A sterile cotton swab was soaked in this suspension and then streaked across Mueller–Hinton agar plates followed. A 2 cm segment of a minocycline/rifampicin-impregnated catheter was embedded in the centre of a Mueller–Hinton agar plate. These experiments were done in triplicate. The inoculum was prepared by placing grown colonies of bacteria in normal saline and the turbidity of the bacterial suspension was adjusted by a spectrophotometer to 0.5 McFarland units. A sterile cotton swab was soaked in this suspension and then streaked across Mueller–Hinton agar plates in three different directions in ambient air. CLSI guidelines were followed. A 2 cm segment of a minocycline/rifampicin-impregnated catheter was embedded in the centre of a Mueller–Hinton agar plate freshly inoculated with one of the four tested original strains, designated as ‘O’ (Original). The agar plates were incubated at 37°C for 72 h, with measurement of zones of inhibition at 24 h. The size of the zones of inhibition for each strain was assessed by measuring the diameter of the clear zone perpendicular to the long axis of the catheter segment. At 72 h, colonies designated as ‘3E’ (3 Edge) were collected from the bacterial lawn just outside the outer edge of the zone of inhibition. Bacterial colonies were specifically collected from this location as they would have conceivably been exposed to minimal amounts of minocycline and rifampicin, and thus have the highest theoretical risk of developing antimicrobial resistance. The 3E strains were then inoculated onto fresh Mueller–Hinton agar plates and exposed, as described above, for another 72 h to fresh minocycline/rifampicin-impregnated catheter segments, with measurement of the zones of inhibition at 24 h. At 72 h, bacterial colonies were collected from bacterial strains at the edge of the bacterial lawn and designated as ‘6E’ (6 Edge). Using the same repeated scheme, we conducted seven 3 day cycles of sequential exposure of organisms to minocycline/rifampicin-impregnated catheters and collected bacterial colonies of strains located at the edge of the corresponding clear zones of inhibition. These bacterial strains were designated as O, 3E, 6E, 9E (9 Edge), 12E (12 Edge), 15E (15 Edge), 18E (18 Edge) and 21E (21 Edge).

MIC determinations

MICs of minocycline, rifampicin, vancomycin and linezolid were determined by using Etest strips purchased from AB BIODISK (Solna, Sweden). These four drugs were specifically tested because (i) minocycline and rifampicin may leach off the surface of impregnated vascular catheters and affect pathogens residing on or adjacent to the catheter, and (ii) since vancomycin is the first-line agent for the treatment of most staphylococcal infections and linezolid is the mainstay of treatment for VRE infections, it would be important to determine whether these two agents would continue to be active after use of minocycline/rifampicin-impregnated catheters. Grown colonies from the edge of the zones of inhibition of bacterial strains designated as O, 3E, 6E, 9E, 12E, 15E, 18E and 21E were placed in normal saline and the turbidity of the bacterial suspension was adjusted by a spectrophotometer to 0.5 McFarland units. A sterile cotton swab was soaked in this suspension and then streaked across Mueller–Hinton agar plates in three different directions in ambient air. After a few minutes, an Etest strip was placed in the middle of the plate using sterile forceps. After incubating the inoculated plates at 37°C for 24 h, the elliptical cut-offs were read according to the manufacturer’s instructions.

Statistical analysis

The mean diameter of the zones of inhibition at baseline and at day 21 were compared by a paired Student’s t-test. The MICs for each organism were analysed over time by means of regression analysis using Stata 8 (Statacorp., College Station, TX, USA). A P value <0.05 indicated a significant difference.

Results

The zones of inhibition of the four organisms did not appreciably change after 21 days of sequential exposure to the impregnated catheters (P > 0.05) as shown in Table 1. The MICs of the antibiotics against the tested organisms are depicted in Figure 1. In general, the MICs of minocycline and rifampicin remained constant, except for the MIC of rifampicin for MRSA (P = 0.01) which increased after 12 days of exposure but remained within the susceptible range (≤1 mg/L). Likewise, the MIC of linezolid increased only for MRSE (P = 0.04) but also remained within the susceptible range (≤4 mg/L). The MIC of vancomycin remained stable.

Discussion

A primary objective of this in vitro study was to assess the antimicrobial efficacy of minocycline/rifampicin-impregnated catheters following sequential bacterial exposure to the catheters.

Table 1. Comparison of mean zones of inhibition (ZI) at baseline versus 21 days, along with standard error of the mean

<table>
<thead>
<tr>
<th>Organism</th>
<th>Mean ZI at baseline (mm)</th>
<th>Mean ZI at day 21 (mm)</th>
<th>P value for paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSA</td>
<td>34.3 ± 3.8</td>
<td>37.3 ± 1.2</td>
<td>0.61</td>
</tr>
<tr>
<td>MRSA</td>
<td>29 ± 4.5</td>
<td>31.7 ± 3.2</td>
<td>0.35</td>
</tr>
<tr>
<td>MRSE</td>
<td>34.3 ± 1.8</td>
<td>35.3 ± 3.5</td>
<td>0.62</td>
</tr>
<tr>
<td>VRE</td>
<td>25.3 ± 0.3</td>
<td>26.3 ± 0.7</td>
<td>0.23</td>
</tr>
</tbody>
</table>

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The zones of inhibition surrounding the antibiotic-impregnated catheter segments remained stable over time for all four tested organisms. Impregnated catheters with an in vitro zone of inhibition ≥15 mm were found to be protective against *S. aureus* colonization both in vivo and in clinical trials. Since the zones of inhibition for all tested organisms remained ≥25 mm throughout the 21 day study period, it is conceivable that these anti-infective catheters may confer clinical protection for at least 3 weeks after catheter insertion.

Another objective of this study was to examine the possibility of selection or evolution of resistant organisms following exposure to minocycline/rifampicin-impregnated catheters. Antibiotic resistance can develop from repeated exposure of organisms to subinhibitory concentrations of antibiotics that fail to eradicate them. Although a group of researchers reported resistance to rifampicin after 10 passages of *S. epidermidis* through subinhibitory concentrations of rifampicin in broth, the same strain did not become resistant after 20 passages through a combination of minocycline and rifampicin. The same authors noted an increase in the MIC of minocycline and rifampicin after 20 serial transfers of *S. epidermidis* and *Escherichia coli* through a combination of both drugs. Another group of researchers observed a lack of emergence of resistance to either minocycline or rifampicin upon exposure of *S. epidermidis, S. aureus, E. coli* and *Enterococcus faecalis* to antimicrobial-impregnated catheters for only 3 days. In our in vitro 21 day study that used actual catheters to simulate a clinical scenario, no organism developed clinically relevant microbiological resistance. Although there was a slight increase in the MIC of rifampicin for MRSA, this increase in MIC did not translate to decreased potency of the antimicrobial-impregnated catheters as evidenced by the stable zones of inhibition throughout the 21 day study period. Furthermore, the slight increase in MIC of rifampicin occurred after 12 days, the affected bacterial strain remained susceptible to minocycline and there was no concurrent increase in the MIC of other tested drugs.

The results of this in vitro study concur with clinical observations. Four year surveillance data in a hospital unit that solely used minocycline/rifampicin-impregnated CVCs did not demonstrate an increase in the prevalence of staphylococci resistant to minocycline or rifampicin when compared both to a historical control and to another hospital unit that solely utilized un-impregnated catheters. Furthermore, resistant organisms were not isolated from patients participating in prospective randomized clinical trials that evaluated the efficacy of minocycline/rifampicin-impregnated catheters. On the contrary, surveillance studies in critical care units using minocycline/rifampicin-impregnated catheters reported a decrease in VRE infections. This potentially beneficial impact could be explained by the ability of clinically protective catheters to reduce the

Figure 1. MICs of various antibiotics for MSSA, MRSA, MRSE and VRE. The y-axis is on a logarithmic scale.
presence of bacteria within the biofilm which acts as a reservoir for antimicrobial-resistant or -tolerant organisms, including those assessed in this study.

In summary, we have demonstrated that minocycline/rifampicin-impregnated catheters maintain their potency against Gram-positive bacteria during sequential exposure over a 21 day period, and do not lead to emergence of drug-resistant bacteria. However, it is essential to continue surveillance of antimicrobial susceptibilities because of the expanded use of anti-infective catheters.

Acknowledgements


Transparency declarations

Saima Aslam, M.D. has no financial conflicts of interest. Rabih O. Darouiche, M.D. has assigned the rights of two patents that describe impregnation of vascular catheters with the combination of minocycline and rifampicin to his employer Baylor College of Medicine, which has executed licensing arrangement with Cook, Inc., for the use of the these two patents.

References