Chelator-Based Catheter Lock Solutions in Eradicating Organisms in Biofilm

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Two different chelator-based antimicrobial catheter lock solutions, Methylene Blue-Citrate-Parabens (MB/CIT) or Minocycline-EDTA-25% Ethanol (MEDTA/25% ETOH), were compared in 2 hour biofilm eradication experiments. Eradication of both mature and immature gram positive, gram negative and fungal biofilms were assessed. MEDTA/25% ETOH was able to fully eradicate all biofilms within 2 hours. MB/CIT was more effective against immature biofilms but was unable to fully eradicate most of the mature biofilms tested.

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The lumen of the central venous catheters (CVC) is an important route to central line associated blood stream infections (CLABSI), and is usually locked with heparin or flushed with saline. However, heparin has been shown recently to stimulate *Staphylococcus aureus* biofilm adherence to the catheter surface (12, 13). Chelators (such as citrate or EDTA) on the other hand, have been shown to have equivalent anti-coagulant/anti-thrombotic activity to heparin with the advantage of disrupting biofilm and enhancing antimicrobial agents that penetrate biofilm such as minocycline (2, 9, 10). Chelator-based catheter lock solutions consisting of either methylene blue with citrate (MB/CIT-Zuragen; Ash Access Technology, Lafayette, IN) or minocycline with EDTA (M-EDTA) have been shown to have activity against organisms embedded in biofilm and were also found to be effective in preventing a CLABSI after a dwell time of at least 24 hours (1, 3, 6, 7, 11). However, a dwell time of 24 hours is not practical as it cannot be accomplished in sick, hospitalized patients requiring different intravenous agents and blood products through various lumens. Previously, we have shown that adding 25% ethanol to M-EDTA would enhance its activity and rapidly eradicate MRSA and *Candida parapsilosis* within 2 hours in immature biofilm (8). In this current study, we are comparing the activity of MEDTA/25% ETOH and MB/CIT against various resistant bacterial and fungal (*Candida* species) pathogens in immature and mature biofilm after 2 hour exposure.

Biofilm was grown on sterile silicone discs (1 cm diameter) following a modified Kuhn’s method (5). Silicone discs were placed into a 24 well tissue culture plate and incubated overnight at 37°C with 1 mL human plasma. The plasma was removed and replaced with 1mL of 5.5x10^5 CFU/mL inoculum of various organisms. For immature biofilm, the plates were incubated for 24hrs at 37°C. For mature biofilm, the plates were incubated for 48 hrs with media replaced after 24 hrs. Inoculum was then removed and discs were washed shaking for 30 minutes in 0.9% sterile saline. After washing, discs were placed in 1mL of lock solution and incubated at 37°C for 2 hrs. The discs were then removed and
placed in 5mL of 0.9% sterile saline and sonicated to disrupt any remaining biofilm. The resulting solution was then quantitatively cultured by making serial dilutions in 0.9% sterile saline and plating on agar plates (TSA+5% sheep blood for all bacterial organisms and Sabouraud Dextrose Agar for yeasts). Challenge organisms were Methicillin Resistant Staphylococcus aureus (MRSA 4798), Vancomycin Resistant Enterococcus faecium (VRE 3868), multidrug resistant (MDR) - Pseudomonas aeruginosa (PS 4689), MDR – Acinetobacter baumannii (An 855), Candida albicans (CA 009-3072) and a clinical strain of Candida glabrata (CG). The lock solutions tested were the quadruple combination MB/CIT (0.05% Methylene blue, 7.0% Sodium citrate, 0.15% Methylparaben and 0.015% propylparaben) and the triple combination MEDTA/25% ETOH (0.1% Minocycline, 3% Calcium EDTA and 25% ethanol). No lock solution (broth) was applied to the controls.

Quantitative recoveries (CFU/disk) from biofilms following exposure to the lock solutions for 2 hours are presented in Figures 1A-C for the gram positive, gram negative and fungal organisms respectively. Reductions in recoveries for MB/CIT versus controls were significant (p<0.05) for A. baumannii at 24 and 48hr, P. aeruginosa at 24 and 48hr, VRE at 24 and 48hr, MRSA at 24hr, C. glabrata at 24hr and C. albicans at 24 and 48hr. There was no significant reduction (p>0.05) against C. albicans 48hr and MRSA at 24hr. MEDTA/25% ETOH gave significant reductions versus controls (p<0.01) against both 24 and 48hr biofilms of all organisms. Reductions in viable organisms for MEDTA/25% ETOH versus MB/CIT treatments were significant (p<0.01) for C. albicans at 24 and 48hr, C. glabrata at 48hr, and MRSA at 24 and 48hr. Trends were present but underpowered to show significance for VRE at 48hr (p=0.07) and A. baumannii at 48hr (p=0.07). It is to note, that the number of organisms recovered in some of the assays is below the lower limit of detection in the serial dilution and quantitative cultures.
Our data show that MEDTA/25% ETOH and MB/CIT were equally effective in eradicating VRE and MDR \textit{P. aeruginosa} within 2 hours in immature biofilm. In addition, these two lock solutions were equally efficacious in rapidly eradicating \textit{Acinetobacter} in immature biofilm. However, MEDTA/25% ETOH was more effective in eradicating MRSA, \textit{Candida albicans} and \textit{Candida glabrata} when compared to MB/CIT in mature biofilm.

MB/CIT has been shown to have activity against organisms embedded in biofilm after 24 hour exposure and Maki, et al, have shown that this solution when locked in for at least 48 hours in hemodialysis patients does significantly decrease the risk of CLABSI (6, 11). However, even in that hemodialysis clinical trial, it was shown that there were breakthrough infections with \textit{Staphylococcus aureus} and gram negative \textit{Bacillus} in the arm that used MB/CIT, which is consistent with our data.

Since staphylococci gram negative organisms (such as \textit{Klebsiella}, \textit{P. aeruginosa}, \textit{Enterobacter} spp., and \textit{E. coli}), and \textit{Candida} species represent more than 70% of organisms causing CLABSI (4), MB/CIT might have limited role in prevention. However, based on our data, MB/CIT might be useful as salvage therapy in CLABSI caused by VRE or \textit{Pseudomonas aeruginosa}.

On the other hand, MEDTA/25% ETOH was highly effective in eradicating all biofilm embedded organisms tested, in this study within two hours of exposure. Its broad spectrum and rapid cidal activity potentially makes it a useful agent for prevention of CLABSI in high risk patients that require heavy usage of the catheter and concurrent short lock time. In addition, MEDTA/25% ETOH may be used in the salvage of indwelling CVC in the setting of CLABSI caused by any organism, whereby there is a mature biofilm that has already been formed. Patients with CLABSI are often hospitalized requiring various intravenous products, and in that setting, a daily hour catheter lock with MEDTA/25% ETOH may provide a rapid and effective salvage solution to the indwelling CVC.
In conclusion, the two tested chelator-based lock solutions are highly effective in eradicating VRE and *P. aeruginosa* embedded in immature and mature biofilm within two hours. For eradication of the majority of organisms that cause CLABSI, such as *Staphylococci*, *Acinetobacter*, and *Candida* species, through a short lock time period, the MEDTA/25% ETOH lock should be further studied in clinical trials to verify its utility in the prevention and treatment of CLABSI. It also remains to be verified, that the complete MEDTA/25% Ethanol lock composition does not impair the functional (mechanical) performance of catheters within which it will indwell, and that contact with catheters will not adversely impact the rapid biofilm eradication properties of the lock.
References:


FIGURE LEGEND:

Figure 1. Median quantitative recovery of organisms from biofilm after 24 and 48 hours exposure to resistant gram positive organisms (A), gram negative organisms (B), and candida (C) followed by 2 hours exposure to control (broth), MB/CIT and MEDTA/25% ETOH lock solutions.

ABBREVIATIONS

MB/CIT = methylene blue with citrate; MEDTA/25% ETOH = minocycline with EDTA and 25% ethanol; MRSA = Methicillin-resistant staphylococcus aureus; VRE = vancomycin-resistant enterococci; PS = P. aeruginosa; AN = A. baumannii; CA = C. albicans; CG = C. glabrata; CLABSI = central line associated blood stream infection.