Zuragen™ Injection, an antimicrobial/antithrombotic solution, is effective against Staphylococcus aureus biofilms

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ABSTRACT

Background: Staphylococcus aureus, a versatile human and animal pathogen, is commonly associated with catheter-related bloodstream infections and has been demonstrated to frequently colonize and form biofilms in medical devices. Since catheter lumens are frequently filled with various bactericidal solutions, this study was performed to test the efficacy of the antimicrobial/antithrombotic solution Zuragen™, a catheter lock solution, against the biofilm-forming pathogen S. aureus.

RESULTS

To determine the efficacy of Zuragen™ and Heparin varying in concentration and preservatives, total CFU from both biofilms and bacteria present in the bulk liquids following 48 h of treatment under static conditions was used as a result parameter. The results are summarized in Figure 3. Our data indicated that increasing Heparin concentrations coincided with reduced biofilm viability (Figure 3) and in preventing microbial colonization and biofilm in catheters. Zuragen™ is an antimicrobial catheter lock solution (CLS) capable of eliminating planktonic bacteria from indwelling devices. Zuragen™ is a mixture of several compounds:

- 20K, 10K, 5K, 1K heparin/parabens
- 20K, 10K, 5K heparin/0.5 x parabens
- 10K, 5K heparin/no preservatives
- 1K heparin/no preservatives
- 10K heparin/no preservatives
- 20K heparin/no preservatives
- 10K heparin/no preservatives
- 5K heparin/no preservatives
- 2K heparin/no preservatives
- 1K heparin/no preservatives
- 0K heparin/no preservatives
- 20K heparin/no preservatives
- 10K heparin/no preservatives
- 5K heparin/no preservatives
- 2K heparin/no preservatives
- 1K heparin/no preservatives
- 0K heparin/no preservatives
- 20K heparin/no preservatives
- 10K heparin/no preservatives
- 5K heparin/no preservatives
- 2K heparin/no preservatives
- 1K heparin/no preservatives

MATERIAL AND METHODS

Bacterial strain and medium used. S. aureus UA4829 (strain RN4220 containing plasmid pPCKG1) was used. This strain was exposed to Sigma antibiotics under flowing conditions. All medium was used for experiments.

Biofilm Formation. Biofilms were grown in flow cells as previously described (Sauer et al., 2005). Medium was adjusted to 0.1 ml/min, resulting in a fluid residence time of 60 minutes. Biofilms were grown under static conditions at 37°C in 5% CO2.

Biofilm Analysis. Biofilms were removed from the following solutions for a period of 48 h: Zuragen™, Saline, heparin (0.05, 0.10 U/ml) containing 1.5% benzyl alcohol as a preservative, Heparin (20,000 U/ml, 10,000 U/ml, 5,000 U/ml, 1,000 U/ml) containing 0.5% parabens and 0.1% benzyl alcohol, Heparin (20,000 U/ml, 10,000 U/ml, 5,000 U/ml, 1,000 U/ml) without preservatives. Image acquisition. Biofilms were imaged using a LVM 510 Meta inverted confocal laser scanning microscope (Zeiss, Heidelberg, Germany) equipped with the LSM 510 Meta image acquisition software (Zeiss). An average of 6 image stacks were acquired 1, 2, 6, 24, 48, and 72 h post initiating flow. Images were taken at random along the length of the flow cell. Each image stack was analyzed using COMSTAT. Changes in biofilm architecture following treatment are shown in Figure 5.

Fig. 1. S. aureus biofilm development over the course of days growing under flowing conditions. Confocal images were acquired (A) 2 hours, (B) 8 hours, (C) 1 day, (D) 5 days, and (F) 4 days post inoculation.

Fig. 2. Biofilm biomass (A) and average and maximum thickness (B) of S. aureus biofilms grown for a period of 4 days under flowing conditions. Biofilm variables were analyzed using COMSTAT.

Effect of Zuragen™ and Heparin on S. aureus biofilm viability

To visualize the effect of Zuragen™ and Heparin varying in concentration and preservatives, confocal images of the S. aureus biofilms were acquired 0 to 60 minutes and after 1, 2, 6, 24, 48, and 72 h of treatment with various lock solutions of treatment with various solutions. The results are summarized in Figure 4. Several biofilm variables (biofilm thickness, etc.) indicative of biofilm architecture were subsequently analyzed using COMSTAT. Changes in biofilm architecture following treatment are shown in Figure 5.

Fig. 3. Effect of catheter lock solutions on S. aureus biofilm viability. Viability (CFU/ml) of S. aureus biofilms (A) and S. aureus present in the supernatant of biofilms (B) after treatment for 48 hours with different tested solutions under static conditions. (C) Log reduction of S. aureus biofilm and total cells (biofilm cells plus S. aureus present in the supernatant of biofilms) after 48 hours treatment with various tested solutions.

Effect of Zuragen™ and Heparin on Staphylococcus aureus biofilms

CONCLUSIONS

Zuragen™ was the most effective of all solutions tested:

- Highest reduction in biofilm viability (Figure 3) 
- Treatment with Heparin for 48 h resulted in the total elimination of all S. aureus cells present in the bulk liquid (Figure 3B) 
- No viable cells were detected in the bulk liquid following treatment with Heparin for 1 and 24 h only resulted in a ~2.4 fold and 1.5 fold reduction in biofilm biomass, respectively. Saline treatment did not affect the biofilm biomass. The findings were confirmed by viability determinations. Treatment with saline resulted in a ~2.4 fold reduction in the biofilm biomass over a period of 3 days after which a plateau was reached. Therefore, all subsequent experiments were carried out using 3-day-old, steady-state biofilms.

Conclusions. To test the efficacy of both Heparin and Zuragen™ on established Staphylococcus aureus biofilms, we first determined the time along the catheter lumen necessary to reach steady-state biofilms with respect to biofilm biomass and biofilm thickness under the conditions tested. This was accomplished by three time point formation after a period of 4 days using confocal microscopy (Fig. 1) and subsequently analyzing the confocal images with respect to biofilm biomass and biofilm thickness by COMSTAT.

Conclusions. After 3 days of treatment, the biofilm biomass increased over a period of 3 days after which a plateau was reached. Therefore, all subsequent experiments were carried out using 3-day-old, steady-state biofilms.

Conclusions. As shown in Fig. 2, the biofilm biomass increased over a period of 3 days after which the biomass appeared to reach a steady-state. The same was also true for both the average and maximum thickness of S. aureus biofilms. The surface area and biofilm biomass increased over a period of 3 days after which a plateau was reached. Therefore, all subsequent experiments were carried out using 3-day-old, steady-state biofilms.

Conclusions. Following 48 h of treatment with various lock solutions, biofilms were stained using a Live/Dead stain to visualize killing of biofilm bacteria (Fig. 6). Bacteria stained in red are dead while bacteria stained in green are viable.

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