The antibiofilm activity of Acetylsalicylic acid, Mefenamic acid, Acetaminophen against biofilms formed by *P. aeruginosa* and *S. epidermidis*

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Abstract

Objective: To evaluate the antibacterial activity of Aspirin, Mefenamic acid and Acetaminophen against Pseudomonas aeruginosa and Staphylococcus epidermidis biofilms.

Methods: The study was conducted AKU Karachi in collaboration with DIHE Karachi from March 2018 to December 2018.Quantitative spectrophotometric method was used to study the reduction and removal of the Pseudomonas aeruginosa and Staphylococcus epidermidis formed biofilms. Statistical tests were performed using Graph Pad Prism software.

Results: Acetaminophen showed maximum biofilm reduction activity against the biofilms formed by Pseudomonas aeruginosa, and Staphylococcus epidermidis. Mefanamic acid showed maximum biofilm removal potential against Pseudomonas aeruginosa, while Aspirin and Mefanamic acid were equally effective in removing biofilms formed by Staphylococcus epidermidis as well.

Conclusion: There is a continuous need to look for non-antibiotic agents for their potential antimicrobial and antibiofilm potential.

Keywords: Antibiofilm activity, Aspirin, Mefenamic acid, Acetaminophen, P. aeruginosa, S. epidermidis biofilms. (JPMA 69: 1493; 2019).**doi:10.5455/JPMA.295488**

Introduction

About 60-80% of all human infections have been found to be associated with biofilm-forming microbes.[1,2] Drug resistance has been frequently attributed to biofilms which are formed by pathogens.[3,4] *Staphylococcus (S.) spp. and Pseudomonas (P.) aeruginosa remain significant causes of biofilm-attributed diseases of contact lenses, sutures, ventilation associated pneumonia, mechanical heart valves, vascular grafts, arteriovenous shunts, endovascular catheter infections, and penile and orthopaedic prosthesis.[3] Moreover, such pathogens have been implicated in causing multitude of hospital-acquired infections, namely the emerging Methicillin-resistant S. epidermidis (MRSE)[5] and multi-drug resistant P. aeruginosa (MDRPa) infections.[6]* Therefore, there is constant need to look for non-antibiotic drugs for their potential antimicrobial and antibiofilm potential.[7] Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used for relieving pain, inflammation and fever.[8] Some reports have shown the antimicrobial activity of NSAIDS against bacteria.[9,10] The current study was planned to evaluate the antibacterial activity of Aspirin, Mefenamic acid and Acetaminophen against P. aeruginosa and S. epidermidis biofilms.

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Materials and Methods

The study was conducted at AKU Karachi in collaboration with DIHE Karachi from March 2018 to December 2018. P. aeruginosa isolate 6 (PA-6) and S. epidermidis isolate 10 (SEP/W-10) from the Immunology and Infectious Disease Research Laboratory, University of Karachi, Karachi, were used after they were previously isolated from infected contact lens and infected wound, respectively.[11-13] Both the isolates were characterised to be dominant biofilm formers.[11-13] The isolates were maintained on Tryptone Soya broth (Sigma-Aldrich) at 37°C.

To assess efficacy of Aspirin, Mefenamic acid and Acetaminophen to reduce and remove P. aeruginosa and S. epidermidis biofilms, quantitative spectrophotometric biofilm reduction and removal method[14] was used to measure the biofilm disinfection and removal efficacy of the NSAIDs against the two isolates. Staining and analysis were done as described previously.[11,12] For biofilm reduction assay, the isolates were inoculated in 5-ml Tryptic Soy Broth (TSB) and grown overnight at 37°C. Subsequently, the cultures were diluted 1:100 in TSB, and 100µl of each diluted culture was pipetted in total 10 wells - two wells for each test agent (Acetylsalicylic acid, Mefenamic acid and Acetaminophen), two for blank (B) and two for control (C) — of a fresh 96-well, non-tissue culture treated microliter plate. One hundred microlitre of each test agent was inoculated in each well, with the exception of blank and control wells, and the plate was

covered and incubated at 37°C for 24 hours.

For biofilm removal assay, microtitre plates were inoculated as mentioned above and incubated at 37°C for 24 hours. After incubation, the plates were washed with sterile water to remove planktonic cells. Subsequently, 200µl of each test agent was inoculated in each well with exception of blank and control wells. Plates were incubated for a period of 1.5 hour at 37°C. After incubation, the plates were washed thrice with Phosphate-buffered saline (PBS) through vigorous pipetting.

For biofilm staining, 125ul of 0.1% crystal violet solution was added to each well and incubated for 10min at room temperature. Following incubation, the plates were washed with PBS, and vigorously tapped on paper towels to remove all the contents and were left to air-dry. Finally, the dye was solubilised by adding 200µl of 95% ethanol to each well of the plate, and incubating the plate for 10-15 minutes at room temperature. Subsequently, contents of each well were mixed by repeated pipetting, and then 125µl of the crystal violet-ethanol solution was transferred from each well to a separate well of a new optically clear flat-bottom 96-well plate. Optical densities (ODs) of each of these 125µl samples were measured at a wavelength 630nm.

Measurement of anti-biofilm efficacy (called percentage removal/reduction) was calculated using equation[12]:

Percentage Reduction/Removal= [(C-B) - (T-B) / (C-B)]*100%

where B = absorbance of blank (no biofilm, no treatment), C = absorbance of control (biofilm, no treatment) and T = absorbance of test (biofilm and treatment).

Statistical significance in biofilm formation in the wells serving as control and treated with NSAID test agents was tested using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. $P<0.05$ was considered significant. The statistical tests were performed using GraphPad Prism software.

Results

Acetaminophen was found to be the most significant in reducing *P. aeruginosa* and *S. epidermidis* biofilm formation, 40% and 25%, respectively. Similarly, Mefenamic acid showed moderate-to-low antibiofilm potential against *P. aeruginosa* and *S. epidermidis* biofilms. Aspirin, however, showed low antibiofilm potential against *P. aeruginosa* biofilms (Table-1), but did not show any activity against *S. epidermidis* biofilms (Table-2).

Aspirin and Mefenamic acid showed appreciable removal of *P. aeruginosa* biofilms (range: 32-45%), while

Table-1: Activity of non-steroidal anti-inflammatory drugs (NSAIDs) against Pseudomonas aeruginosa biofilm.

Test agents	Biofilm Reduction	P-value	Biofilm Removal	P-value
Control	0	-	0	-
Aspirin	5	< 0.05*	32	0.0001*
Mefanamic Acid	7	< 0.05*	45	0.0001*
Acetaminophen	40	0.0001*	0	>0.05

*Statistically significance difference in biofilm formation between control and test agents.

Table-2: Activity of non-steroidal anti-inflammatory drugs (NSAIDs) against Staphylococcus epidermidis biofilm.

Test agents	Biofilm Reduction	P-value	Biofilm Removal	P-value
Control	0	-	0	-
Aspirin	0	>0.05	28	0.0001*
Mefanamic Acid	20	0.0001*	28	0.0001*
Acetaminophen	25	0.0001*	25	0.0001*

*Statistically significance difference in biofilm formation between control and test agents.

Acetaminophen showed no activity against *P. aeruginosa*. Conversely, Aspirin, Mefenamic acid and Acetaminophen showed modest removal of *S. epidermidis* biofilms (range: 25-28%).

Discussion

The study noted that acetaminophen was the most significant agent in reducing P aeruginosa and S epidermidis biofilm formation. This finding is significant because the NSAIDs are in frequent use to treat pain and inflammation and account for the ever-increasing biofilm-associated infections.[15] A possible mechanism has been proposed by reducing the gene expression of the cAMP-EGF1 pathway. Diclofenac acid has shown to inhibit biofilm formation.[16] Our findings support earlier reports which have stated that antibiotics may act in a synergistic manner when used with NSAIDs,[17,18] thereby synergising the prophylactic purpose of antibiotics when used perioperatively.[18] Moreover, NSAID-mediated inhibition of the biofilm formation by decreasing adhesion of bacteria may make a case to use NSAIDs to further decrease risk of infections postoperatively.

Though the reported increased efficacy of antibiotics, when combined with NSAIDS against P.aeruginosa, is encouraging.[19] However, the current study could not ascertain why acetaminophen showed little activity against biofilm removal, which might require additional investigation. In addition, it has been reported that *S. epidermidis* shows moderate bacterial adherence when water-soluble NSAIDs are added in its growth medium.[20] Our study supports the earlier findings and enhances

knowledge about the actual biofilm reduction and removal potential of NSAIDs.

Conclusion

Results emphasised and validated that there is a constant need to look for non-antibiotic drugs for their potential antimicrobial and antibiofilm potential.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: All consumables were funded by the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, for projects falling under the Introduction to Research Module.

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