Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

In vitro efficacy of bismuth thiols against biofilms formed by bacteria isolated from human chronic wounds

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Keywords
antibiotic, antimicrobial, biofilm, bismuth, chronic wound, MRSA.

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2011/0568: received 4 April 2011, revised 17 June 2011 and accepted 15 July 2011

doi:10.1111/j.1365-2672.2011.05110.x

Abstract

Aims: The purpose of this study was to evaluate the antimicrobial efficacy of thirteen bismuth thiol preparations for bactericidal activity against established biofilms formed by two bacteria isolated from human chronic wounds.
Methods: Single species biofilms of a *Pseudomonas aeruginosa* or a methicillin-resistant *Staphylococcus aureus* were grown in either colony biofilm or drip-flow reactors systems. Biofilms were challenged with bismuth thiols, antibiotics or silver sulfadiazine, and log reductions were determined by plating for colony formation.
Conclusions: Antibiotics were ineffective or inconsistent against biofilms of both bacterial species tested. None of the antibiotics tested were able to achieve >2 log reductions in both biofilm models. The 13 different bismuth thiols tested in this investigation achieved widely varying degrees of killing, even against the same micro-organism in the same biofilm model. For each micro-organism, the best bismuth thiol easily outperformed the best conventional antibiotic. Against *P. aeruginosa* biofilms, bismuth-2,3-dimercaptopropanol (BisBAL) at 40–80 μg ml^{-1} achieved >7·7 mean log reduction for the two biofilm models. Against MRSA biofilms, bismuth-1,3-propanedithiol/bismuth-2-mercaptopyridine *N*-oxide (BisBDT/PYR) achieved a 4·9 log reduction.
Significance and Impact of the Study: Bismuth thiols are effective antimicrobial agents against biofilms formed by wound bacteria and merit further development as topical antiseptics for the suppression of biofilms in chronic wounds.

Introduction

Mounting evidence indicates that microbial biofilm formation may contribute to chronicity of some wounds (Bjarnsholt *et al.* 2008; Davis *et al.* 2008; James *et al.* 2008). These are wounds that do not heal within 3 months. Examples include the following: some diabetic foot ulcers, venous leg ulcers and pressure ulcers. Chronic wounds can become painful and stressful for the patient when nerves become damaged. Failure to heal can result in amputation. These wounds are reported to affect 2–6·5 million Americans each year (Singer and Clark 1999), most above the age of 60, and cost about $10 billion in treatment expenses (Bickers *et al.* 2004; Kuehn 2007).

The use of systemic antibiotics and topical antibiotics or antiseptics as part of wound healing therapeutic regimens remains controversial (White *et al.* 2006; Hinchliffe *et al.* 2008; Lipsky and Hoey 2009; O'Meara *et al.* 2010). If the biofilm hypothesis of wound infection is correct, the inconclusive efficacy of antimicrobial agents in chronic wounds could be explained by the well-known protection from killing by antimicrobial agents that is afforded to bacteria in biofilms (Costerton *et al.* 1999; Stewart and Costerton 2001). An important step towards improving antimicrobial activity in chronic wounds will be to use biofilm methods to evaluate efficacy.

Bismuth thiols are the combination of bismuth nitrate with a lipophilic thiol containing compound (Domenico *et al.* 2001; Veloira *et al.* 2003). This complex of bismuth

with a thiol compound has been described as increasing the solubility and antimicrobial properties of bismuth (Domenico *et al.* 1997; Veloira *et al.* 2003). Bismuth thiols enhance *in vitro* immune response against *Klebsiella pneumoniae* and *P. aeruginosa* (Domenico *et al.* 1999; Wu *et al.* 2002; Alipour *et al.* 2010). They also inhibit exopolysaccharide production (Domenico *et al.* 2001; Badireddy *et al.* 2008b), resulting in decreased biofilm (Domenico *et al.* 2001; Badireddy *et al.* 2008a). Additionally, bismuth thiols exhibit synergy with antibiotics (Veloira *et al.* 2003). These properties indicate that bismuth thiols could make excellent antimicrobials.

If biofilms pose a barrier to healing in chronic wounds, then effective antibiofilm therapies may aid healing in these cases. Using established methods, we determine which of many possible bismuth thiols could be most effective in the treatment of biofilms formed by bacteria isolated from human chronic wounds.

Materials and methods

Chronic wound isolates

Methicillin-resistant *Staphylococcus aureus* 10943 (MRSA) and *Pseudomonas aeruginosa* #215 were obtained from the Southwestern Regional Wound Center (Lubbock, TX, USA). MRSA was grown statically overnight in tryptic soy broth (TSB; Becton Dickinson, Franklin Lakes, NJ) at 37°C, and *P. aeruginosa* was grown overnight in TSB at 37°C and 150 rev min^{-1} in 250-ml baffled Erlenmeyer flasks. Stock cultures were maintained at −70°C using Microbank vials (Pro-Lab diagnostics, Austin, TX, USA).

Antibiotics and antimicrobial agents

The antibiotics, imipenem monohydrate, cefepime HCl (U. S. Pharmacopeia, Rockville, MD, USA), rifampicin, vancomycin, tobramycin, ampicillin (Sigma-Aldrich chemical, St. Louis, MO, USA), ciprofloxacin (Fluka Biochemika, Sigma-Aldrich chemical), daptomycin (Cubist pharmaceuticals, Lexington, MA, USA), minocycline and amikacin (MP Biomedicals, LLC; Solon, OH, USA) were used for antibiotic susceptibility testing of biofilms.

Bismuth thiol preparations (Table 1) were provided by Microbion Biosciences (Bozeman, MT, USA), and 5-mg ml^{-1} stocks was fresh prepared daily in DMSO. Silver sulfadiazine (Spectrum Chemical, Gardena, CA) was prepared as 1% slurry in 0·1% peptone or PBS.

Colony biofilm susceptibility

Colony biofilms were prepared according to Anderl *et al.* (2000), with modifications. Briefly, overnight bacterial cultures were diluted in TSB to an OD_{600} of 0·05, and one 10-μl drop is placed onto sterile, black, polycarbonate membrane filters (K02BP02500; GE Water and Process Technologies, Trevase, PA). Prior to inoculation, the filters were applied to tryptic soy agar (TSA; Becton Dickinson) modified to contain only 10% of the usual amount of pancreatic digest of casein and papaic digest of soybean (10% TSA). Biofilms were grown for 24 ± 2 h, before being challenged with antimicrobial for 18 h.

To determine biofilm antibiotic challenge levels, minimum inhibitory concentrations for the antibiotics including azithromycin, tetracycline and ampicillin/Sulbactam 2 : 1 were determined using E-test strips (AB Biodisk, Solna, Sweden). Colony biofilms were challenged with antibiotics, bismuth thiols and combinations of these treatments. The treatments were prepared in Mueller Hinton agar (Oxoid, Basingstoke, UK) plates amended with the desired treatment. For daptomycin challenge, we used BBL Mueller Hinton II agar (Becton Dickinson) supplemented with calcium chloride to a final concentration of

Table 1 Bismuth thiol complexes used in this work

Abbreviation	Bismuth thiol (Bi : thiol molar ratio)
BisEDT	Bismuth-1,2-ethanedithiol (2 : 3)
BisBAL	Bismuth-2,3-dimercaptopropanol (2 : 3)
BisERY	Bismuth-dithioerythritol (2 : 3)
BisTOL	Bismuth-4-methyl-1,2-benzenedithiol (2 : 3)
BisBDT	Bismuth-2,3-butanedithiol (2 : 3)
BisHPT	Bismuth-1-mercapto-2-propanol (1 : 3)
BisBDT/PYR	Bismuth-2,3-butanedithiol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisBAL/PYR	Bismuth-2,3-dimercaptopropanol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisEDT/PYR	Bismuth-1,2-ethanedithiol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisTOL/PYR	Bismuth-4-methyl-1,2-benzenedithiol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisPDT/PYR	Bismuth-1,3-propanedithiol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisERY/PYR	Bismuth-dithioerythritol/Bismuth-2-mercaptopyridine *N*-oxide (2 : 1 : 2)
BisHPT/EDT	Bismuth-1-mercapto-2-propanol/Bismuth-1,2-ethanedithiol (1 : 1 : 1)

≈50 mg l^{-1}. Susceptibility assays were performed in at least duplicate.

Drip-flow biofilm susceptibility

Colony biofilms were used as a screening tool. Drip-flow biofilms, which are more complicated to prepare, were used to confirm effects of selected treatments. Drip-flow biofilms were prepared according to Xu *et al.* (1998), with modifications. Briefly, the overnight culture of *P. aeruginosa* was diluted tenfold ($OD_{600} \approx 1$), and MRSA was diluted fivefold ($OD_{600} \approx 1$), with 10 ml of 0·1% peptone and used to inoculate each channel of the drip-flow reactor (BioSurfaces Technologies Corp., Bozeman, MT, USA). Dakin fully frosted slides (no. 2958-001; Thermo Scientific, Portsmouth, NH, USA) were placed in each channel to provide a growth surface for the biofilms. After 2 h static incubation, biofilms were grown for 72 h at a flow rate of 10 ml h^{-1} per channel using L/S 13 tubing, with masterflex pumps (Cole Parmer, Vernon Hills, IL) operating at 2·61 rev min^{-1} with TSB diluted tenfold for MRSA or 100-fold for *P. aeruginosa*. These growth conditions were based on a published protocol (Buckingham-Meyer *et al.* 2007). Growth and inoculation were performed at 37°C.

Drip-flow biofilms were challenged for 24 h with selected bismuth thiols, selected antibiotics or 1% silver sulfadiazine. Antibiotics were used at 100 times the MIC, and bismuth thiols were applied at 40, 80, 200 and 500 μg ml^{-1}. Antibiotics, silver sulfadiazine and bismuth thiol treatments for *P. aeruginosa* drip-flow biofilms were prepared in 20 ml of 0·1% peptone and in PBS for *S. aureus* treatments. Treatments for drip-flow biofilms were applied to slides removed from the reactor and laid flat in a Petri dish. Susceptibility assays were performed in at least duplicate.

Viable cell enumeration

Biofilms were dispersed into 0·1% peptone. Colony biofilms were transferred with the membrane and vortexed at maximum for 20 s, and drip-flow biofilms were dispersed by scraping with a Teflon policeman and agitation by hand. Dispersed biofilms were homogenized for 30 s at ≈11 500 rev min^{-1} (Ultra Turrax T25 S1; IKA Labortechnik, Wilmington, DE), serially diluted in 0·1% peptone water and enumerated by the drop method (Hoben and Somasegaran 1982; Herigstad *et al.* 2001) onto tryptic soy agar using 10-μl drops. Colonies were counted and marked daily with colonies appearing within 4 days counted. Bismuth thiols were neutralized with the addition of 2 g l^{-1} reduced glutathione (Acros Organics, Mullica Hill, NJ) to the dispersal medium and also to the plates used for enumeration.

Results

Colony biofilm susceptibility

Several antibiotics were tested against bacteria in colony biofilms to provide comparisons for the efficacy of bismuth thiols and to guide the selection of agents to be tested subsequently in combination with bismuth thiols. MICs were determined for planktonic *P. aeruginosa* and MRSA, and colony biofilms of each were challenged with ten or one hundred times the MIC. Colony biofilms of these strains were very tolerant to antibiotic treatment (Table 2). Ciprofloxacin killed 3·5 log CFU within *P. aeruginosa* colony biofilms, while rifampicin killed approximately 1 log within MRSA colony biofilms. The other antibiotics were not effective in killing the colony biofilms. *P. aeruginosa* was resistant to azithromycin and tetracycline, and these antibiotics were not included in any further testing.

Colony biofilms of *P. aeruginosa* and MRSA were challenged with several different bismuth thiols to identify those with the greatest bactericidal activity against biofilms (Table 3). Efficacy ranged widely from no effect to several log reductions. The most efficacious bismuth thiol for each bacterial species performed better than the most efficacious antibiotic for that micro-organism. Overall, MRSA colony biofilms were less susceptible than those of *P. aeruginosa* (Fig. 1). Bismuth thiols effective against one species did not usually display comparable efficacy, at the same dose concentration, against the other micro-organism ($R^2 = 0{\cdot}15$ for the data plotted in Fig. 1).

To identify antibiotics that yield synergy with bismuth thiols, we combined antibiotics with either 80 μg ml^{-1} BisEDT used to challenge *P. aeruginosa* colony biofilms or 200 μg ml^{-1} BisEDT for MRSA colony biofilms. Combinations were considered to be synergistic if the log kill achieved by the combination was greater than the sum of the log kills achieved by the bismuth thiol and antibiotic separately. Tested under the conditions used in these studies, the combination of amikacin with BisEDT resulted in synergy (Table 4) when challenging *P. aeruginosa* colony biofilms, while with MRSA colony biofilms, no synergy was found with any of the combinations, and only the combination with rifampicin exhibited even marginal efficacy (Table 4). Based on this information, *P. aeruginosa* and MRSA were challenged with other bismuth thiols combined with amikacin or rifampicin, respectively (Table 5). Six combinations of amikacin and bismuth thiols were effective against *P. aeruginosa*, four of which were synergistic combinations with BisBAL, BisBDT, BisTOL/PYR and BisHPT/EDT. For MRSA, two were effective with combinations of rifampicin and

Table 2 Minimum inhibitory concentrations (MIC) of antibiotics using E-test strips and the effect of antibiotics on viability of bacteria in colony biofilms

Antibiotic	MIC (μg ml^{-1})	Treatment	Log kill	*n*	SD
P. aeruginosa					
Ciprofloxacin	0·38	100 × MIC	3·5	2	0·1
		10 × MIC	3·6	3	0·1
Tobramycin	2·0	10 × MIC	0·2	3	0·1
Cefepime	12·0	10 × MIC	0·1	3	0
Imipenem	0·38	10 × MIC	0·0	3	0·2
Azithromycin	>256·0	–	–		
Tetracycline	>256·0	–	–		
Amikacin	16·0	100 × MIC	1·5	3	0·2
		10 × MIC	0·3	3	0·1
MRSA					
Ampicillin	16	10 × MIC	−0·7	3	0·1
Ampicillin/Sulbactam 2 : 1	6	–	–		
Minocycline	2·5	100 × MIC	−0·03	3	0
		10 × MIC	−0·29	3	0·1
Vancomycin	1	10 × MIC	−1·1	3	0·1
Rifampicin	0·006	100 × MIC	0·9	3	0·1
		10 × MIC	1·1	3	0
Daptomycin	0·125	320 × MIC	−0·4	4	0·1
		10 × MIC	−0·73	4	0·1

Table 3 The effect of bismuth thiols on the viability of bacteria in colony biofilms

Bismuth thiol	Log kill	*n*	SD
P. aeruginosa colony biofilms treated with 80 μg ml^{-1}			
BisEDT	1·2	3	0·5
BisBAL	>7·2	3	1·5
BisERY	1·7	2	0·1
BisTOL	−0·3	2	0·0
BisBDT	−0·1	2	0·0
BisBDT/PYR	1·0	3	2·0
BisBAL/PYR	5·8	2	0·8
BisEDT/PYR	2·5	2	0·1
BisTOL/PYR	3·7	2	2·3
BisPDT/PYR	2·1	2	0·6
BisERY/PYR	4·6	2	0·3
BisHPT/EDT	>5·9	3	0·8
MRSA colony biofilms treated with 200 μg ml^{-1}			
BisEDT	0·2	3	0·3
BisBAL	2·4	4	1·8
BisERY	0·1	2	0·2
BisTOL	−0·1	2	0·1
BisBDT	0·6	3	0·1
BisBDT/PYR	4·9	5	0·9
BisHPT	0·2	2	0·2
BisBAL/PYR	1·6	2	0·2
BisEDT/PYR	2·8	2	1·2
BisERY/PYR	2·7	2	1·6
BisHPT/EDT	1·2	2	0·3

BisBAL or BisBDT/PYR being synergistic. Some of the bismuth thiol/antibiotic combinations tested were antagonistic.

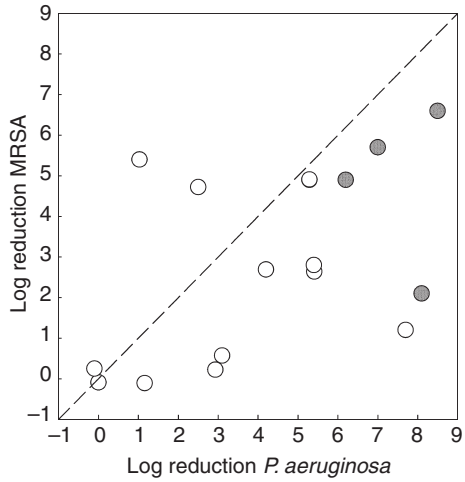

Figure 1 Lack of correlation between efficacy of various bismuth thiols against the two microbial species tested in colony biofilm (open symbols) and drip-flow biofilm (closed symbols) models. The correlation coefficient for the linear regression of these data was R^2 = 0·15. The fact that the data fall mostly below the line of equality (dashed line) shows that MRSA biofilms were generally less susceptible than *P. aerguinosa* biofilms.

Drip-flow biofilm susceptibility

The most effective bismuth thiols for each microorganism were tested in a second *in vitro* biofilm model system. The drip-flow biofilm reactor is a continuous flow system (Goeres *et al.* 2009). Bismuth thiols performed similarly or better against biofilms grown in

Table 4 Effect of BisEDT in combination with antibiotics on the viability of bacteria in colony biofilms

Antibiotic	Log kill	*n*	SD	Additive log kill*
P. aeruginosa 80 μg ml^{-1} BisEDT				
None	1·2	3	0·5	n/a
10 × MIC Ciprofloxacin	2·0	3	0·8	4·8
100 × MIC Ciprofloxacin	2·8	2	0·3	4·7
10 × MIC Tobramycin	1·4	3	0·5	1·4
10 × MIC Amikacin	3·0	3	0·7	1·5
100 × MIC Amikacin	>6·6	3	0·6	2·7
10 × MIC Cefepime	0·2	3	0·1	1·3
MRSA 200 μg ml^{-1} BisEDT				
None	0·2	3	0·3	n/a
10 × MIC Minocycline	0·4	3	0·2	−0·1
100 × MIC Minocycline	0·4	3	0·3	0·25
10 × MIC Daptomycin	0·0	3	0·3	−0·5
10 × MIC Rifampicin	0·8	3	0·1	1·34
100 × MIC Rifampicin	1·0	3	0·3	1·1
10 × Vancomycin	0·3	3	0·4	−0·9
10 × Ampicillin	0·1	3	0·1	−0·5

*Sum of the log kill of each agent individually for comparison.

Table 5 The effects of antibiotics combined with bismuth thiols on the viability of colony biofilms

Combination treatment (Bi : thiol)	Log kill	*n*	SD	Additive log kill*
P. aeruginosa challenged with 100× amikacin plus bismuth thiol				
40 μg ml^{-1} BisBAL	5·3	2	1·4	2·2
80 μg ml^{-1} BisBDT	>7·7	2	0·4	1·4
200 μg ml^{-1} BisBDT/PYR	>7·3	2	1·0	6·8
500 μg ml^{-1} BisTOL/PYR	>7·7	2	0·4	5·3
80 μg ml^{-1} BisERY/PYR	>6·7	2	1·0	6·1
40 μg ml^{-1} BisHPT/EDT	>8·3	2	0·4	7·1
MRSA challenged with 100× MIC rifampicin plus indicated bismuth thiol				
500 μg ml^{-1} BisBAL	>5·9	3	0·9	4·2
80 μg ml^{-1} BisBDT/PYR	>7·4	2	0·2	6·3

*Sum of the log kill of each agent individually for comparison.

the drip-flow reactor compared to the colony biofilm (Table 6, Fig. 2). For both bacterial species, the most effective bismuth thiol outperformed or matched the most effective antibiotic or silver sulfadiazine treatment (Table 6).

Most of the bismuth thiols exhibited the anticipated dose–response behaviour of increasing effectiveness with increasing concentration (Fig. 2). The effectiveness of BisEDT/PYR against MRSA was opposite, declining from 4·7 log CFU kill at 80 μg ml^{-1} to 0·6 log CFU kill at 500 μg ml^{-1}.

Table 6 The effects of bismuth thiols and selected comparison treatments on the viability of bacteria in drip-flow biofilms

Treatment	Log kill	*n*	SD
P. aeruginosa			
100 × MIC ciprofloxacin	0·8	3	0·3
100 × MIC amikacin	>6·7	3	1·0
100 × MIC tobramycin	2·6	2	0·1
1% silver sulfadiazine	1·0	4	0·5
40 μg ml^{-1} BisBAL	>8·1	3	0·3
40 μg ml^{-1} BisBDT/PYR	6·2	3	0·4
40 μg ml^{-1} BisHPT/EDT	6·6	3	0·5
MRSA			
100 × MIC rifampicin	0·4	3	0·2
100 × MIC vancomycin	−0·5	3	0·1
1% silver sulfadiazine	4·1	4	1·6
40 μg ml^{-1} BisBAL	2·1	3	0·7
40 μg ml^{-1} BisBDT/PYR	>4·9	3	1·1

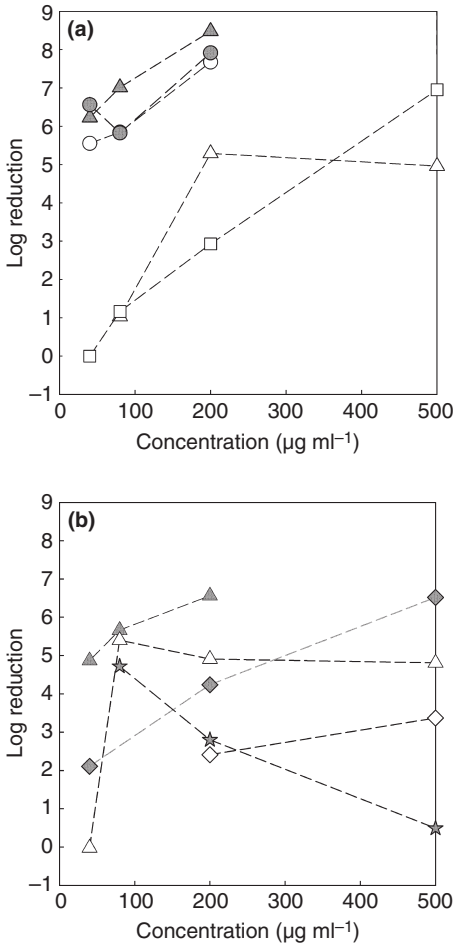

Figure 2 Dose response of bismuth thiols against colony biofilm (open symbols) and drip-flow biofilm (closed symbols) for *P. aeruginosa* (a) and MRSA (b). BisBAL, (diamond); BisBDT/PYR, (triangle); BisEDT, (square); BisHPT/EDT, (circle); BisEDT/PYR, (star).

Discussion

Choice of *in vitro* models

The *in vitro* biofilm models selected for this investigation were intended to simulate two physical aspects of the wound environment: low fluid shear and proximity to an air interface. The colony biofilm model also captures the expected geometry of nutrient delivery in a wound: carbon and nitrogen sources emanating from host tissue (agar in the model) with oxygen supplied from the opposite side of the biofilm. One reason to use two different models is that every model gives somewhat different results. Potential therapies that perform well in more than one *in vitro* model probably have a better chance of affording robust efficacy in real-world applications. We chose to work with strains of *P. aeruginosa* and *S. aureus* because these two bacteria are commonly identified in human chronic wounds (Fazli *et al.* 2009; Frank *et al.* 2009; Malic *et al.* 2009; Körber *et al.* 2010; Melendez *et al.* 2010; Thomsen *et al.* 2010). In reality, chronic wounds are colonized by mixed populations of bacteria (Dowd *et al.* 2008; James *et al.* 2008; Kirketerp-Møller *et al.* 2008; Frank *et al.* 2009). A logical next step for testing of bismuth thiols would be to evaluate their efficacy in a mixed species model (Percival *et al.* 2008; Sun *et al.* 2008; Hill *et al.* 2010).

Antibiotic susceptibility of biofilms

Antibiotics were ineffective or inconsistent against biofilms of both bacterial species tested. None of the antibiotics tested were able to achieve >2 log reductions in both biofilm models. Tobramycin, cefepime and imipenem showed little efficacy against *P. aeruginosa* biofilms. While ciprofloxacin was somewhat effective against colony biofilms of *P. aeruginosa* (log reduction 3·5), it performed poorly against drip-flow biofilms formed by the same strain (log reduction 0·8). Amikacin was the most effective antibiotic for *P. aerguinosa* biofilms overall. Against MRSA biofilms, ampicillin, minocycline, vancomycin and daptomycin were completely ineffective. Only rifampicin at 100× MIC managed a fraction of a log reduction. These results show that these two *in vitro* biofilm models captured the antibiotic tolerance that is a hallmark of the biofilm mode of existence (Stewart and Costerton 2001). We conclude that these are reasonable models for evaluating the antibiofilm efficacy of alternative therapeutic agents.

Bismuth thiol efficacy against biofilms

The thirteen different bismuth thiols tested under the conditions used in this investigation achieved widely varying degrees of killing, even against the same micro-organism in the same biofilm model. For example, against *P. aeruginosa* colony biofilms, BisTol had no effect (log reduction −0·3), whereas BisBAL left no surviving bacteria (log reduction > 7·2). Similarly against MRSA colony biofilms, BisTOL had no effect (log kill −0·1), whereas BisBDT/PYR recorded a 4·9 log reduction. The differential efficacy of particular bismuth thiols between the two different micro-organisms is evident in Fig. 1. What these results mean is that the choice of bismuth thiol needs to be tailored to the particular application.

For each micro-organism, the most effective bismuth thiol easily outperformed the most effective conventional antibiotic. For example, against *P. aeruginosa* biofilms, the most effective antibiotic was amikacin at 100 × MIC (1600 μg ml^{-1}), which achieved a mean log reduction (averaging colony biofilm and drip-flow biofilm results) of 4·1 BisBAL at 40 to 80 μg ml^{-1} achieved a >7·7 log reduction for the same assays. Against MRSA biofilms, rifampicin at 100× MIC (0·6 μg ml^{-1}) achieved a mean log reduction of only 0·7 BisBDT/PYR at 40–200 μg ml^{-1} achieved a mean log reduction of 4·9 The most effective bismuth thiols also outperformed 1% silver sulfadiazine, a benchmark antimicrobial in treating wound infection (Akiyama *et al.* 1998; Bjarnsholt *et al.* 2007). At 200 μg ml^{-1}, a typical bismuth thiol delivers approximately 0·5 mmol l^{-1} bismuth. At 1% silver sulfadiazine, the concentration of silver is more than 50 times higher on a molar basis (28 mmol l^{-1}). These results indicate that bismuth thiols may be competitive with existing antimicrobials for the treatment of bacteria in microbial biofilms.

We tested for synergy between antibiotics and bismuth thiols. The only clear example of synergy was between amikacin and several bismuth thiols used against *P. aeruginosa* biofilms (Table 5).

Two bismuth thiols stand out as particularly interesting based on our results: BisBAL and BisBDT/PYR. Numerous studies have found BisBAL to be a promising compound in a wide variety of applications. It has been reported to inhibit microbial growth on coated stents (Zhang *et al.* 2005), reduce drinking water biofilms (Codony *et al.* 2003) and reduce virulence of *P. aeruginosa* (Wu *et al.* 2002). BisBAL reportedly has low concentration MBCs for *B. cepacia* complex (Veloira *et al.* 2003) and a low MIC for *E. coli*. Huang and Stewart examined the effects of BisBAL on preformed biofilm of *P. aeruginosa* (Huang and Stewart 1999) and found that BisBAL at MIC concentration was able to kill biofilm cells of *P. aeruginosa* biofilms. In the current study, BisBAL was most effective against biofilms formed by *P. aeruginosa* and was the second most effective bismuth thiol against MRSA biofilms.

The other bismuth thiol of interest emerging from this study was one that has not been described in the literature before, BisBDT/PYR. This bismuth thiol was the best performing antimicrobial against established MRSA biofilms, which were highly recalcitrant to antibiotics. It also exhibited good activity against *P. aeruginosa* biofilms. The advanced efficacy demonstrated in this study suggests that additional investigation of a more expansive series of bismuth thiols against a broader range of disease-related biofilms is warranted.

Acknowledgement

This work was supported by Montana Board of Research and Commercialization Technology grant no. 09-20. Co-author Baker wishes to disclose a financial interest in the bismuth thiol technology, which has been licensed by Microbion Corporation. Dr Baker's contributions to the work included the development of the experimental hypothesis and selection and synthesis of the bismuth thiols used. Microbion's commercial interests did not influence the experimental design or interpretation of results.

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