eP288
ePoster Viewing
Basic science: biofilm pathophysiology

SETTING UP A NADH OXIDASE (NOX)-BASED ASSAY TO QUANTIFY ANTIBIOTIC ACTIVITY IN PNEUMOCOCCAL PLANKTONIC CULTURES AND BIOFILMS.

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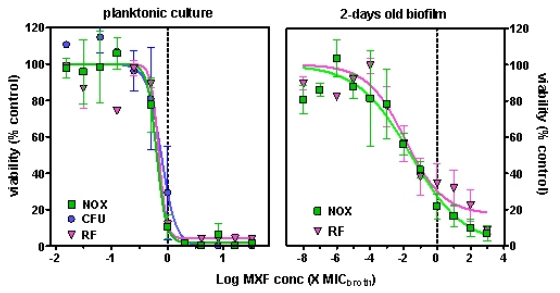
Objectives: Measuring the release of a soluble cytosolic enzyme is a widely used approach in eukaryotic cells cytotoxicity studies that is rarely applied to bacteria although it could help for the rapid screening of bactericidal antibiotics under various conditions of culture. NADH oxidase (NOX), a soluble flavoprotein allowing reoxidation of NADH using oxygen rather than pyruvate, functions as defence against oxidative stress in anerobic bacteria. In *S. pneumoniae*, an anaerobic but aerotolerant pathogen, NOX is also essential for expression of virulence and competence [Mol Microbiol. 1999, 34:1018-28]. As NADH does not permeate through membranes, its oxidation when added to the medium will be a measure of NOX release from dying bacteria. Our objective was to assess the method using moxifloxacin (MXF) as test antibiotic against *S. pneumoniae* growing in biofilms or in broth in comparison with the resazurin reduction assay (Lett. Appl. Microbiol. 2008,49:249-54) for biofilms and planktonic bacteria and CFU counting (planktonic bacteria only).

Methods: *S. pneumoniae* ATCC49619 was used throughout. Planktonic cultures were made in CA-MHB+ 5% lysed horse blood (96-wells plates; initial OD_{620nm}=0.1). Biofilms (attached to the bottom of the wells) were obtained after 2 days of culture with the same medium plus 2% glucose. MXF activity was measured after 24 h of incubation with full concentration-response curves used to fit a Hill equation and determine pertinent pharmacodynamic parameters. Clarithromycin was used as a bacteriostatic control. Release of NOX was assessed by measuring the decrease in absorbance (OD_{340nm}) of added NADH (0.17mM) over 30 min at 37°C. Reduction of resazurin to resorufin (RF) was measured as the increase in fluorescence signal [excit. 560nm; emiss. 590nm] over 1h (planktonic cultures) or 72 h (biofilm) incubation with 0.001% resazurin. CFU were enumerated by plating assay.

Results: The Figure shows the change in viability as a function of MXF concentration as determined by each of the 3 types of assay. Numerical values of the key parameters of the Hill equations with statistical analyses are presented in the Table. Although some statistically significant differences were noted, values were always close from each other. No decrease of NADH concentration was seen when added to the medium of biofilms exposed to clarithromycin at concentrations up to 1000 x its MIC in broth.

Conclusion: The assay of NOX in the medium allows quantifying MXF bactericidal activity against *S. pneumoniae* in both planktonic and biofilm cultures, and yields results similar to the resazurin assay (planktonic and biofilm) and CFU counting (planktonic). As this NOX assay can be completed in 30 min and is amenable to performance in various media, it may offer advantages for the rapid screening of bactericidal antibiotics in different environments and/or culture conditions.



Change in viability of *S. pneumoniae* ATCC49619 exposed to increasing concentrations of moxifloxacin (MXF) for 24 h, as determined using the NADH oxidase assay (NOX)[a], the resazurin redox assay (RF)[b] and colony forming units counting (CFU; planktonic cultures only)[c]. Data are expressed in percentage of the corresponding control (no drug added).

[a,b,c] see notes in Table

PARAMETER	planktonic			biofilm	
	NOX [a]	RF [b]	CFU [c]	NOX [a]	RF [b]
E_{max} [d]	1.97 ± 3.70 (A ; a)	4.02 ± 2.87 (A ; a)	0 ± 5 .38 (A)	6.65 ± 5.36 (A ; b)	19.39 ± 6.27 (B ; b)
EC_{50} [e]	0.64 ± 1.11 (A ; a)	0.77 ± 1.17 (A ; a)	0.70 ± 1.16 (A)	0.04 ± 2.29 (A ; a)	0.01 ± 2.60 (A ; a)
E_{MIC} [f]	10.4 ± 3.9 (A ; a)	11.9 ± 2.8 (A ; a)	31.2 ± 5.7 (B)	24.3 ± 3.5 (A ; b)	19.4 ± 1.5 (A ; b)

[a-c] Viability in comparison with control (no antibiotic added) assessed by: [a] NADH oxidase assay (% of NADH remaining in medium); [b] resazurin redox assay (% of conversion to resorufin); [c] colony counting assay (% of number of colonies)

[d] residual viability (in % of control value) as extrapolated using the Hill equation for an infinitely large MXF concentration

[e] concentration in MXF (in log_{10} of its MIC in broth [0.125 mg/L]) causing a change in viability at half way between E_{min} and E_{max} (50% effect)

[f] residual viability as intrapolated using the Hill equation for a concentration of moxifloxacin equal to the MIC in broth (0.125 mg/L)

Statistical analysis (one-way ANOVA with Tukey post-test or unpaired t-test with Welch's correction): values with different letters are significantly different from each other ($p<0.05$). Upper case letters: comparison of the results from the different assays in one model (NOX *vs* RF [*vs* CFU]); lower case letters: comparison between the same type of assay (NOX or RF) between culture conditions (planktonic *vs* biofilm).