

Activity evaluation of supramolecular compound based on N-acetylcysteine for the treatment of *P. aeruginosa* biofilm

Guerini M.,^{1*} Grisoli P.¹, Perugini P.¹

¹ University of Pavia, Department of Drug Sciences, Pavia, Italy
paola.perugini@unipv.it

Abstract:

Cystic Fibrosis (CF) is a genetic pathology causing mucus obstruction, neutrophil-dominated airway inflammation and bacterial infection (1). *Pseudomonas aeruginosa*, a gram-negative bacillus, produces a biofilm, that prevents the penetration of antibiotics. Furthermore, the microorganisms inside are increasingly aggressive (2) (3). This work focused on the evaluation of effectiveness of a supramolecular compound based on N-acetylcysteine (NACESOLTM), against *P. aeruginosa* biofilm. The prevention activity was evaluated both by microbial assay and by Scanning Electron Microscopy (SEM) associated with the X-Ray Spectroscopy (EDS) to determine the elemental composition of surface films in the SEM (4).

For the production of the biofilm, *P. aeruginosa* ATCC 10145 was cultured overnight in TSB under aerobic conditions at 37°C; then a stainless-steel disk was immersed in the bacterial suspension for 48h at 37°C. To be able to determine the actual presence of the biofilm on the disk, a count of the bacterial suspension after mechanical stresses and sonication of the stain-less steel surface was performed. After that different weights of NAC and NACESOLTM were put in a biofilm suspension previously formed to having different concentrations of N-acetylcysteine (0.5 mg/ml, 2 mg/ml, 4 mg/ml). For each analysis a control was made without active. After 24 hours the suspension is removed from the incubator. Results of the biofilm eradication assays are expressed as percentage values of biofilm reduction using a non-treated metal disk as a control.

Free NAC at 0.5 mg/ml reduces the number of colonies by 19.42% +/- 4.28%, at 2 mg/ml the reduction percentage is the 20.87%, and at 4mg/ml the reduction percentage is 89.52 +/-5.83% .

NACESOLTM at 0.5 mg/ml of NAC is able to reduce the number of colonies by 51.41 % +/- 22.10, at 2 mg/ml the reduction percentage is 68,98% +/-24,30%, and at 4mg/ml the reduction percentage is 48.00 +/-31.14% .

The antioxidant activity of NAC and NACESOLTM was evaluated through their capability of scavenging DPPH radical.

The comparison between free NAC and NACESOLTM show that the antioxidant activity of the NAC is independent of its crystalline or amorphous structure: in fact, NACESOL has an antioxidant activity comparable to the free NAC. To conclude, through the high resolution of the SEM it was possible to have a clear view of the bacteria inside the biofilm, the thickness, the morphology of the same and its gradual eradication with the increase of the concentration of active. We demonstrate also the effective increase of the biological activity of the NAC / α -CD / Resveratrol. Moreover, the DPPH assay demonstrate that the antioxidant activity of NAC and of the multicomposite is the same, and therefore that the active in the multicomposite does not lose its activity.

References:

1. Hartl D. (2012) J. of Cystic Fibrosis. 11, 363–382.
2. Bjarnsholt T. (2009). Pediatric Pulmonology. 44, 547–558.
3. Hentzer M. (2001). J. of bacteriology. 5395–5401.
4. Calheiros L., Gomes J. M. (2017). Scanning. 1-77.