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N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review

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Abstract. – OBJECTIVE: Biofilms are microbial communities consisting of bacteria, extremely capable to self-reproduce on biological surfaces, causing infections. Frequently, these biofilms are resistant to classical antibacterial treatments and host immune response. Thus, new adjuvant molecules are mandatory in clinical practice. N-acetylcysteine (NAC), a precursor to the antioxidant glutathione, has been investigated for its effectiveness both in inhibiting biofilm formation and in destroying developed biofilms. The aim of our study was to conduct a systematic literature review of clinical trials involving NAC as adjuvant treatment to eradicate pre-formed mature biofilms and to inhibit new biofilm production.

MATERIALS AND METHODS: A careful analysis of the Medline was conducted and eight studies were selected according to the following criteria: site of infection, kind of bacteria, design of the research, dose of the treatment, administration, biological effects and results. We fixed an arbitrary scale of scores from 0 (lowest score) to 5 (highest score) for each criterion and a threshold value of 3.

RESULTS: The studies analyzed, with score over 3, suggested a potential role for NAC as adjuvant molecule in the treatment of bacterial biofilms, with an excellent safety and efficacy profile. NAC, in combination with different antibiotics, significantly promoted their permeability to the deepest layers of the biofilm, overcoming the problem of the resistance to the classic antibacterial therapeutic approach.

CONCLUSIONS: Overall, these results are encouraging to a more widespread clinical use of NAC, as adjuvant therapy for microbial infections followed by biofilm settle, which may occur in several body districts, such as the vaginal cavity.

Key Words:
N-acetylcysteine, Biofilm, Mucolytic, Resistance, Adjuvant treatment.

Introduction

The biofilm is a multicellular microbial community of one or more microorganisms extremely capable to self-reproduce on biological surfaces[1].

From the first and reversible contact of an individual bacterium with a surface, biofilm development proceeds with a strong surface association, colony formation, biofilm maturation in an extracellular polysaccharide matrix, and its dispersal from the site of attachment into the environment[2-4]. In other words, a biofilm is a sort of structurally complex ecosystem that allows bacteria to survive to inhospitable conditions, becoming responsible of infections in different organs and tissues[5].

Antibiotics represent the traditional pharmacological approach to eradicate biofilm-producing bacteria. However, they are often ineffective because of their slow or incomplete penetration into the deepest layers of biofilm[6]. Antibiotic resistance of bacteria in the biofilm contributes to the chronicity of infections such as those associated with implanted medical devices[6-8].

Thus, the control of biofilm growth is a really challenging and striking target of medical research.

The use of N-Acetylcysteine (NAC) has been proposed as alternative pharmacological approach to control bacterial biofilm growth in human diseases. Indeed, several *in vitro* studies reported that NAC decreases biofilm formation by a variety of bacteria[9-14]. Moreover, NAC may reduce the production of extracellular polysaccharide matrix[15] while promoting the disruption of mature biofilm[16,17].

N-acetylcysteine is a precursor to the antioxidant glutathione, involved in the Reactive Oxygen Species (ROS) balance and homeostasis[18]. Due to its safety profile, NAC is widely used in medical practice via inhalation, oral and intravenous routes[19,20].

In order to assess NAC safety and efficacy profile in disrupting bacterial biofilms, we conducted a systematic literature review of clinical

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trials involving NAC as adjuvant treatment to eradicate pre-formed mature biofilms and to inhibit new biofilm production.

Materials and Methods

A search of the electronic medical literature databases Medline was conducted, from http://www.ncbi.nlm.nih.gov/pubmed. The search was limited to texts in English. As shows the flowchart in Figure 1, candidate articles were identified by searching for those that included the keywords *N-acetylcysteine*, *biofilm* and *bacteria*. The selected studies (n = 36) were evaluated and 28 were discarded (see Figure 1 for the details). On the basis of the eight resultant studies we performed a systematic review with respect to the following criteria: site of infection, kind of bacteria, design of the research, dose of the treatment, administration route, biological effects and results. An arbitrary scale of scores from 0 (lowest score) to 5 (highest score) for each criterion was fixed, considering as our target of interest the vaginal mucosa and its relevance in relation to the sites of infection and bacteria investigated in the trials presented in this review. A threshold score of 3 was set up and only the studies that exceeded this value were considered really effective and valid.

Results

After the screening of titles and abstracts, and using the established criteria, eight studies were selected for inclusion in the systematic review. Starting from these studies a scoring matrix was laid out (Table I). Below we provided a description of the studies that exceeded the threshold

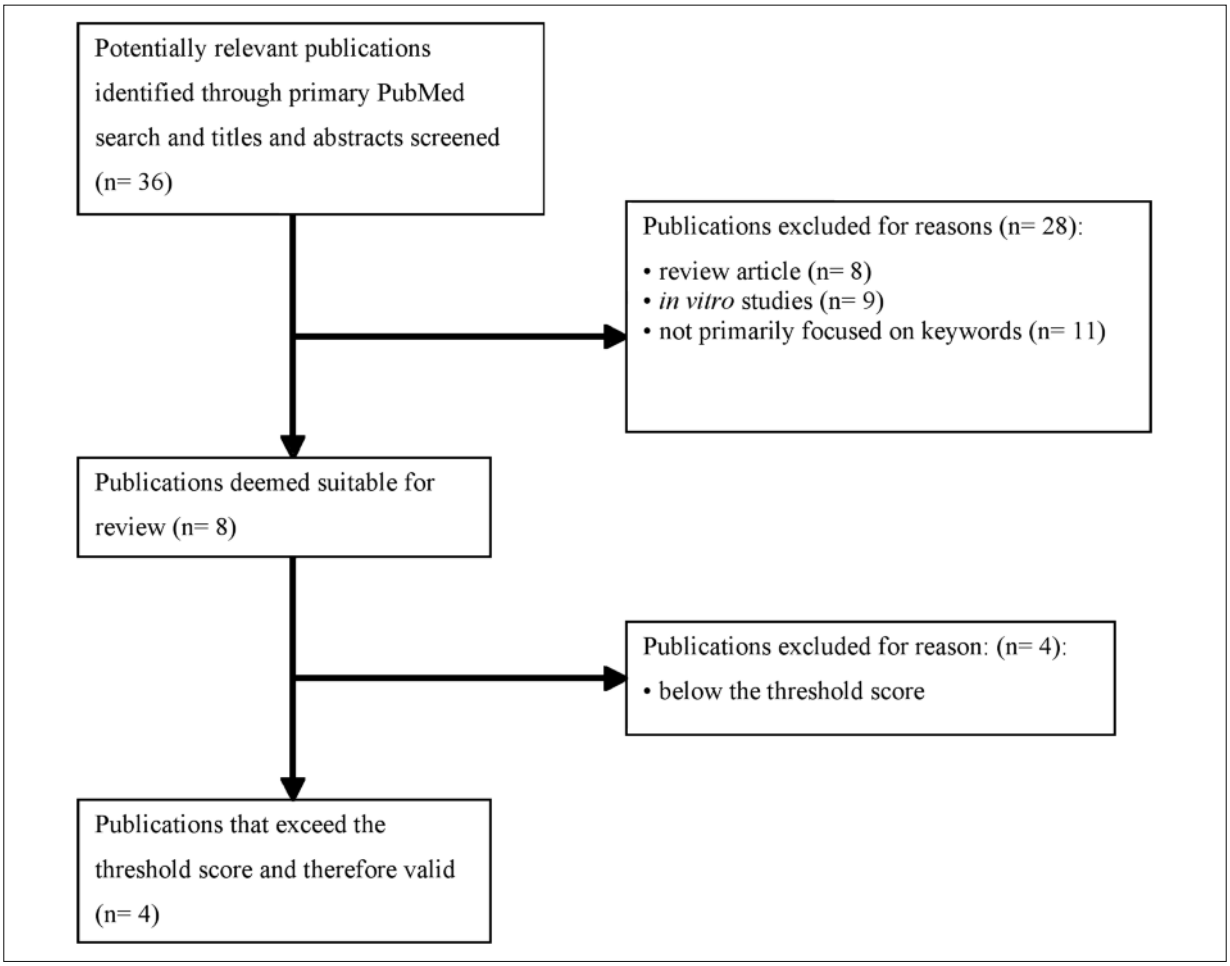

Figure 1. Flowchart showing selection of articles included in the review.

Table I. Scoring matrix of clinical studies of NAC use against bacterial biofilms.

Study	Site of infection	Bacteria	Design	Dose	Administration	Biological effects	Results (%)	Total score	Average score
Zala G (1994)	Gastric mucosa (3)	*H. pylori* (0)	Randomized controlled trial (5)	2 × 600 mg (5)	Oral (2)	Reduction of gastric barrier mucus thickness (3) Reduction of mucus viscoelasticity		18	2.6
Garbuz AK (2005)	Gastric mucosa (3)	*H. pylori* (0)	Controlled clinical trial (3)	400 mg (4)	Oral (2)	Reduction of gastric barrier mucus thickness (3) Increase of permeability of antibiotics	50% (2)	17	2.4
Cammarota G (2010)	Gastric mucosa (3)	*H. pylori* (0)	Randomized controlled trial (5)	600 mg (4)	Oral (2)	Eradication of pre-formed mature biofilms (5) Overcoming antibiotic resistance	65% (4)	23	3.3
Karbasi A (2013)	Gastric mucosa (3)	H. pylori (0)	Randomized controlled trial (5)	600 mg (5)	Oral (2)	Reduction of mucus viscoelasticity (3) Increase of permeability of antibiotics	70% (4)	22	3,1
Macchi A (2006)	Upper respiratory tract (0)	*S. aureus* (3) *S. pyogenes* *S. pneumoniae* *H. influenzae*	Clinical trial (2)		Intramuscular injections/ Aerosol (0)	Eradication of pre-formed mature biofilms (5)	87.5% (5)	15	2.1
Aslam S (2007)	Hemodialysis catheter (5)	*S. aureus* (5) *S. epidermidis*	Pilot clinical trial (2)	Solution (4)	Catheter retention (4)	83% (5)	25	3.6	
El-Feky MA (2009)	Ureteral stent (5)	S. aureus (5) *S. epidermidis* *E. coli* *K. pneumoniae* *P. aeruginosa* *P. vulgaris*	Research article (0)	2-4 mg/ml (2)		Inhibition of biofilm production (5) Eradication of pre-formed mature biofilms	94-100% (5)	22	3.1
Drago L (2013)	Orthopedic implant (5)	*S. aureus* (5) *P. aeruginosa*	Research article (0)			Demolition of biofilm (5)	50%-20% (2)	17	2.4

Score from 0 to 5; threshold 3; Four valid study among 8 (50%). Total score is the sum of the individual scores arbitrarily assigned from 0 to 5; 3 is the threshold value above which the study is considered valid. Valid studies that exceed the threshold are shown in bold.

value and, therefore, were considered scientifically valid. These studies refer to gastric mucosa and catheters, sites prone to bacterial infections that degenerate in the production of biofilms, as well as vagina that was precisely chosen as the target of the study.

Helicobacter Pylori and Gastric Mucosa

H. pylori is the main colonizer of the human stomach, by overcoming gastric acidity and peristalsis, and bypassing host immune response[21]. This pathogen is clearly involved in biofilm formation, and it plays an important role in the resistance to antibacterial therapy[22]. Anyway, relatively little is known about how *H. pylori* triggers the process of biofilm formation[23]. N-acetylcysteine has been demonstrated effective in destroying biofilm, due to its mucolytic properties[24,25] and its bacteriostatic behavior[14].

Two studies matched with our criteria and threshold score. Firstly, we examined the study by Cammarota et al[5], in which 40 patients who had previously failed *H. pylori* treatment were assigned randomly to receive (group A) or not (group B) 600 mg *per os* N-acetylcysteine before a culture-guided antibiotic regimen. *H. pylori* was eradicated in 13 of 20 (65%) group A participants and 4 of 20 (20%) group B participants.

In a randomized double-blinded clinical trial by Karbasi et al[26] 60 *H. pylori* positive patients who were suffering from dyspepsia were included. They were divided into two groups, both of them received pantoprazole 40 mg, ciprofloxacin 500 mg and bismuth subcitrate 120 mg. Experimental group (30 cases) received 600 mg of NAC besides three-drug regimen. Control group received placebo. *H. pylori* infection was eradicated in 21 (70%) and 17 (60.7%) patients in experimental and control groups, respectively.

In both these studies, NAC was able to reduce gastric barrier mucus thickness and mucus viscoelasticity, thus inhibiting biofilm production and eradicating pre-formed mature biofilms. Moreover, NAC bypassed the problem of antibiotic resistance, increasing the permeability of the antibiotics into the deepest layers of biofilm and improving the results of the classic drug therapy with antimicrobial molecules.

Vascular Catheters

Vascular catheter infections due to biofilm-embedded bacteria are difficult to eradicate without removing the infected device. This causes a strong impact on morbidity, mortality, duration of stay, and overall cost of health care[27-31]. The most used therapeutic strategies involve the administration of antibiotic solutions, even if rarely they lead to satisfactory results[31]. Thus, other pharmacological approaches are required. The mucolytic molecule N-acetylcysteine, with an excellent safety profile, is able to decrease biofilm formation by a variety of bacteria, reduce the production of extracellular polysaccharide matrix and encourage the disruption of mature biofilms[15-17,32]. The study we considered, as it matched with our threshold value, is by Aslam et al[7]. In a pilot clinical trial, they demonstrated that NAC, in association with tigecycline, acts synergistically in the treatment of catheter-associated biofilm, developed by *Staphylococcus (S) aureus* and *S. epidermidis*, microorganisms commonly associated with vascular catheter-related infections[27]. The solution of NAC/tigecycline consistently and significantly decreased viable biofilm-associated bacteria in respect to control.

Ureteral Stents

Frequently ureteral stents are the target of obstruction, migration, encrustation, stone formation and biofilm development[33]. Several approaches have been proposed to prevent biofilm formation, such as coating with silver o antiseptics[34]. N-acetylcysteine, although it is not an antibiotic, shows remarkable antibacterial properties, as it may disrupt mucus disulphide bonds and reduce the viscosity of secretion. El-Feky et al[8] studied the effects of ciprofloxacin and N-acetylcysteine, alone and in combination, against *S. aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The association ciprofloxacin/NAC showed the highest inhibitory effect on biofilm production as well as the strongest disruption of pre-formed biofilms, in respect to both to NAC and the antimicrobial drug alone.

Discussion

Bacteria can exist both as individual, planktonic organisms and complex, integrated communities called biofilms. Biofilm is a sophisticated network of pathogens living within protective extracellular polymeric substances[35]. Bacterial resistance and survival are greatly augmented in biofilm, so that both chemical (antibiotics, disinfectants) and biological (viruses, protists) antimicrobial agents may be ineffective to eradicate them entirely[3]. There-

fore, biofilm formation has a negative impact on the effectiveness of infection treatment. For this reason, new pharmacological approaches are desirable, in order to prevent biofilm formation, eradicate mature pre-formed biofilms and increase the permeability of antibiotics, so overcoming the resistance phenomenon.

N-acetylcysteine (NAC) is a molecule derived from the amino acid cysteine, commonly used as antioxidant and free-radical scavenging, because it increases cellular production of glutathione[36]. Moreover, being a mucolytic agent, it is able to dissociate mucin disulphide bonds and other disulphide bond cross-linked gel components to reduce viscosity[37].

NAC is generally safe and well tolerated even at high doses, with a highly favorable risk/benefit ratio and a low rate of adverse events[24].

Several studies have shown that NAC decreases biofilm formation, inhibits bacterial adherence, reduces the production of extracellular polysaccharide matrix, and the cell viability of a variety of Gram-negative and Gram-positive bacteria[13,15,17,32,38,39].

To the best of our knowledge, we conducted the first systematic literature review about the role of NAC in inhibiting and/or destroying bacterial biofilms. On the basis of chosen criteria, such as site of infection, kind of bacteria, design of the research, dose of the treatment, administration, biological effects and results we selected 36 studies. From these 36 publications, 28 were rejected because they were review articles, *in vitro* studies or because they were not primarily focused on the main target of this review.

Thus, we analyzed the resulting eight studies: six clinical trials on patients (three of them randomized controlled trials, the others controlled or pilot clinical trials) and two *in vivo* research articles on vascular and ureteral catheters. We fixed an arbitrary scale of scores from 0 to 5 for each criterion and a threshold value of 3. According to these settings, we selected just 4 studies with a score over the chosen threshold. Therefore, we focused on these publications for our systematic review.

In two randomized controlled trials, respectively from Cammarota et al[5] and Karbasi at al[26], pretreatment with NAC, before an antibiotic therapy against *H. pylori*-mediated biofilms, showed reduction of gastric barrier mucus thickness, reduction of mucus viscoelasticity, increase of permeability of antibiotics with overcoming of drug resistance and eradication of pre-formed mature biofilms.

The other two studies, with an over 3 score and then scientifically valid, were from Aslam et al[7] and El-Feky et al[8]. They were *in vivo* studies on vascular and ureteral catheters, which described the efficacy of NAC in association with typical antimicrobial drugs, in inhibiting biofilms produced by several bacteria such as *S. aureus*, *S. epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*.

Both these publications[7,8] have reinforced the role of NAC as biofilm-dissolving molecule, with excellent results in terms of catheter retention, statistically significant in respect with the treatment with the antibiotics alone.

Conclusions

Overall, even if these studies are really encouraging, they are still very few. Larger studies and, in particular randomized controlled trials, will be necessary to investigate this anti-biofilm role of NAC even toward other biological districts, seat of bacterial infections that lead to biofilm formation, like vaginal mucosa, often colonized by *Candida albicans*, *Gardnerella vaginalis*, *Atopobium vaginae* and other microorganisms[40,41]. It is well known that bacterial biofilms promote vaginosis, vaginitis and other gynecologic infections that are not responsive to antibiotic therapies[42,43]. Moreover, antibiotic-resistant bacterial biofilm arrangement was frequently observed onto intrauterine devices[44]. NAC efficacy in respect to these pathological conditions is still unclarified. Interestingly, Shahin et al[45] demonstrated that oral administration of NAC in association to progestin therapy, on pregnant women with bacterial vaginosis, reduced preterm labor risk without collateral effects for both the mother and the fetus. Studies in this direction are, therefore, desirable and should be encouraged.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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