The Effect of N-Acetylcysteine (NAC) on bacteria isolated from Patients

with Chronic Obstructive Pulmonary Disease (COPD) Hayder Fadhel * Mohammad Sabri ** Ala Hussain Abbase Haider**

College of medicine- Babylon University

Abstract

Twenty patients with COPD have been included in this study who were admitted at Mirjan teaching hospital for Internal medicine, where the disease diagnosed by the physicians according to the criteria of chronic bronchitis with productive cough in a period of three successive months in each of two consecutive years in absence of identifiable causes of sputum production with their age group ranged from 45 up to 86 years at a period from January up to February 2007.

N-acetylcysteine was performed to detect its effect on the growth of twenty bacterial isolates, 90% of these isolates had no growth in the presence of this material, whereas, 10%gave positive growth even in the presence of this substance.

تأثير مادة ن-أسيتيل سيستين على البكتريا المعزولة من مرضى الانسداد الرئوي المزمن

الخلاصة

تم في هذه الدراسة شمول عشرين مريضا من المرضى المصابين بمرض الانسداد الرئوي المزمن في مدينة الحلة والذين تم إدخالهم إلى مستشفى مرجان التعليمي للأمراض الباطنية ، حيث أن مرضهم تم خيصه من قبل أطباء الباطنية والصدرية هناك وبالاعتماد على معايير تشخيص التهاب القصبات المزمن والذي يتميز بوجود سعال منتج ولفترة ثلاثة أشهر متتالية لسنتين متعاقبتين وبغياب الأسباب الأخرى الواضحة لإنتاج القشع ، تراوحت أعمار المرض المشمولين بالدراسة بين ٤٥ و ٨٦ سنة وكانت فترة الدراسة بين كانون الثاني شباط ٢٠٠٧. تم في هذه الدراسة عمل تجربة بيان تأثير مادة ن- أسيتيل سستين (N-acetylcysteine) لبيان تأثيرها على نمو ٢٠ عزلة بكتيرية تم أخذها من هؤلاء المرضى ، حيث بينت الدراسة أن ٩٠% من هذه العزلات لم تنمو بوجود هذه المادة بينما فقط ١٠% من هذه العزلات نمت على الرغم من وجود هذه المادة.

Introduction :

N-Acetyl-L-Cysteine (NAC) is an altered form of the amino acid cysteine which is commonly found in food and synthesized by the body, and it breakdown of the mucous [4]. NAC is used in the medical treatment of chronic bronchitis, cancer, and paracetamol

intoxication where it is one of the smallest drug molecule in use which has antibacterial properties because this molecule is a thiol-containing antioxidant that disrupts the disulphide bonds in mucous and competitively inhibit the utilization of cysteine [6 ; 10]. Likewise, NAC is effective in the treatment of liver failure due to causes other than acetaminophen poisoning like ; hepatitis, and other drug toxicity, also supplementation with NAC has been shown to reduce the proliferation of

certain cells lining the colon and may reduce the risk of colonic cancer in people with recurrent polyps in the colon [2]. NAC exhibits direct and indirect antioxidant properties where it`s free thiol group is capable of interacting with the electrophilic group of reactive oxygen (RO) which leads to the intermediate formation of NAC thiol with NACdisulphide as a major end product, also NAC exerts indirect antioxidant effect related to its role as a reduced glutathione precursor (GSH) [1]. Furthermore, NAC has an effect in lowering the intrabronchial bacterial growth particularly in patients with chronic bronchitis [9], on the same manner, [10] states that the use of NAC as a drug has a negative effect on bacterial adhesive capacity, especially in S.pneumoniae and H.influenzae, and the rate of positive sputum culture will be low

compared with those who do not use NAC as a medicine among COPD patients [11].

Materials and Method :

Patients :

Twenty patients with COPD have been included in this study who were admitted at

Mirjan teaching hospital for Internal medicine, where the disease diagnosed by the Physicians according to the criteria of chronic bronchitis with productive cough in a period of 3 successive months in each of 2 consecutive years in absence of identifiable causes of sputum production with their age group ranged from 45 up to 86 years at a period from January up to February 2007.

Method :

Sputum sample has been obtained from COPD patients by ordinary method and then cultured on ordinary media to show the bacterial growth on these media and recultured the grown bacteria in the presence of NAC to detect the effect of this substance on the growth of these bacteria, this chemical material was prepared by dissolving 0.1gm of this NAC in 10ml distilled water (1%) and put in a sterile plain tube according to the following procedure :

1. After obtaining the sputum from the COPD patients, a mixture of the sputum and normal saline had been prepared.

2. About 0.5ml of this mixture was obtained and mixed with 0.5 ml of NAC (1%) and incubated for 2 hrs.

3. The final mixture had been transferred in Brain heart infusion broth (BHIB) and incubated at 37 Cº for 24 hrs.

4. The results were scored according to the presence and absence of the bacterial growth.The positive result (growth) had been recultured on additional media (Blood and MacConky agars) to detect the type of growth and effect of NAC.

Result :

This study showed the effect of N-acetylcysteine (NAC) on 20 bacterial isolates, it was seen that all 8 isolates of M.catarrhalis had lost their growth when be grown in media containing NAC, only one isolate of H.influenzae tested had a growth in the presence of NAC on chocolate agar, whereas 80% of these isolates lost their growth also, all 3 isolates of S.pneumoniae had no growth in the

presence of NAC , the single isolate of K.pneumoniae and Acinetobacter exhibited no growth with NAC, the single isolate of P.aeruginosa resisted NAC presence and showed growth on both blood and chocolate agars, whereas the single isolate of V.streptococci had no growth with NAC, and the study showed that (90%) of the isolates tested showed no growth with NAC, and only (10%) of the isolates had growth in vitro, as shown in table (1).

Discussion :

Indeed, N-acetylcysteine (NAC), is used in the medical treatment of chronic bronchitis, cancer, and paracetamol intoxication [10], and it is one of the smallest drug molecule in use that has antibacterial properties, where the molecule is thiol-containing antioxidant that disrupts disulfide bonds in mucous and competitively inhibits the amino acid cysteine utilization as shown by [6] , also, NAC exhibits direct and indirect antioxidant properties and it's free thiol group is capable of interacting with the electrophilic of reactive oxygen (RO), where this interaction with RO leads to the intermediate formation of NAC thiol with NAC disulphide as a major end product and NAC exerts an indirect antioxidant effect related to its role as a reduced glutathione precursor (GSH) [1]. Besides, [9] observed that the ratio of significant intrabronchial bacterial growth was lower in patients with chronic bronchitis who were treated with oral NAC medication

Table (1) : The Effect of N-Acetyl cysteine (NAC) on Bacterial Sp. isolated From COPD patients

No.	Type of bacterial isolate	No.	Growth with NAC	No growth with NAC
1.	M.catarrhalis	8	0	8
2.	H.influenzae	5	1	4
3.	S.pneumoniae	3	0	3
4.	P.aerugenosa	1	1	0
5.	K.pneumoniae	1	0	1
6.	Acinetobacter spp.	1	0	1
7.	V.streptococci	1	0	1
	Total	20	2 (10%)	18 (90%)

than in patients without this therapy, and in contrast to the present study in which the NAC was as a chemical material in vitro to test its effect on some bacterial isolates recovered from COPD patients, whereas the study of [10] used NAC as a medicine for some COPD patients and was shown by their study that the rate of positive culture was low with NAC use and NAC had a negative effect on bacterial adhesive capacity of S.pneumoniae and H.influenzae with negative cultures, whereas the cultures were positive without NAC treatment. The current study showed that all 8 isolates of *M.catarrhalis* had a negative growth on both blood and chocolate agars, and this may be attributed to the fact that NAC can reduce the attachment of bacteria to pharyngeal epithelial cell and possibly by removing a layer of ruthenium red-positive extracellular material from the epithelial cells and the effect of NAC on bacteria and bacterial biofilms that showed a response to NAC which may facilitate efficient use of this material as biofilms inhibitor [11], likewise, it has been stated by [8] that NAC was previously used as a mucolytic compound in chronic bronchitis has also antioxidative effect and it influences the intrabronchial bacterial colonization and addition of daily 600 mg NAC lead to a significantly higher bacterial eradication rate and treatment with NAC may reduce the number ,duration of acute exacerbation episodes and influence lung function.

On the same manner, [10] noticed that the presence of NAC during growth of *H.influenzae* and *S.pneumoniae* were significantly reduced their adherence ability to human oropharyngeal epithelial cells in vitro and the effects were dose dependant and NAC affects the adhesive capacity which lowered the bacterial binding for *H.influenzae* and *S.pneumoniae*, also, it had been shown by [5] that the production of pyocyanin enhances *P.aeruginosa* virulence, and many of pyocyanins have cytotoxic effects on human cells which result from its ability of redox cycle where pyocyanin directly accepts electrons from reduced nicotine amide dinucleotide (NADH) or (NADPH) with subsequent transfer to O_2 generating reactive oxygen (RO), reduced glutathione (GSH) which is an important cellular antioxidant that contributes to the regulation of redox- sensitive signaling system and loss of GSH could be due the to pyocyanin induced (H_2O_2) formation. However, overproduction of catalase can partially prevent the pyocyanin- mediated decline in cellular GSH, also pyocyanin oxidized other thiol-

containing compounds, cysteine and NAC and thus GSH may enhance pyocyanin- induced cytotoxicity by functioning as alternative source of reducing equivalents for pyocyanin redox cycling and this may appear the cause of the resistant *P.aeruginosa* to

the effect of NAC. Moreover, [7] pointed that the NAC reduced the adhesion of *K.pneumonia* and it decreases the extracellular polysaccharides(EPS) production by the bacteria by changing its surface character and as a result of the exposure to NAC by changing the EPS

, the bacteria become unable to form large microcolonies and only single cell or small microcolonies were formed which changed the texture of the biofilm formed, and the same mechanism was applied to *Acinetobacter spp.* Which was also affected by NAC in vitro by affection of EPS which was decreased as a result of exposure to NAC and also NAC can reduce the adhesion of the bacteria and it was less resistant than the *K.pneumoniae*.

In addition , NAC reduced cigarette smoke-induced abnormalities in the Polymorph nuclear leukocytes (PMN), alveolar macrophages and epithelial cells), and NAC could also render oropharyngeal bacteria aspirated from the oropharynx less able to adhere to mucous or epithelial cells in the lower airways as reported by [3].

Thus, N-acetylcysteine showed a great effect in vitro on most of the bacterial isolates being tested in this study by inhibiting their growths.

References :

1. Auroma, O. I. ; Halliwell, B. ; Hoey, B. M. ; and Butler, J. (1989) . *Free. Radic. Biol. Med.* , 6 : 593-597.

2. Ben-Ari, Z. ; Vaknin, H. and Tur-kaspa, R. (2000). *Hepatogastroenterology*., 47 : 786-789.

3. Clancy, R.L. ; pang, G. ; Dunkley, M. ; Taylor, D. C. and Cripps, A. W. (1995). Immunol. Cell Biol. , 73 : 414-417.

4. Grandijean, E. M. ; Brethet, P. ; Ruffmann, R. and Leuenberger, P. (2000). *Clin.Ther.* , 22 : 209-221.

5. Malley, Y. Q. O. ; Reszka, K. J. ; Septiz, D. R. ; Denning, G. M. and Britigan, A. E. (2004). *Am.J.Physiol.Lung Cell Mol.* , 287 : L 94-L 103.

6. Naszal, B. ; Visky, D. and Kraszani, M. (2000). J. Med. Chem. , 43 : 2167-2182. 7. Olofsson, A. C. ; Hermansson, M. and Elwing, H. (2003). Appl. Environ. Microbiol., 69(8) : 4814-4822.

8. Reinchenberger, F.and Tamm, M. (2002).*Pneumonologie*. , 56(12) : 793-797.

9. Riise, G. C. ; Larrson, S. ; Larrson, P. ; Jeansson, S. and Andersson, B. A.

(1994). *Eur. Respir. J.* ,7 : 94-101.

10. Riise, G. C. ; Qvarfordt, I. ; Larsson, S. ; Eliasson, V. and Andersson, B. A (2000). *Respiration.* , 67 : 552-558.

11. Zheng , C.H. ; Ahmed, K. ; Rikitomi, N. ; Martinez, G. and Negatake, J.(1999). Microbiol. Immunol. , 43 : 107-113.