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Therapeutics effect of *N*-acetyl cysteine on mustard gas exposed patients: Evaluating clinical aspect in patients with impaired pulmonary function test

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Summary
Aims: Long-term prescription of *N*-acetyl cysteine (NAC) may be effective in diseases caused by active radicals of oxygen species. The aim of this study was to determine the effect of 2- and 4-month administration of NAC (1800 mg daily) on mustard induced bronchiolitis obliterans.
Methods and materials: In a double blind clinical trial, 144 patients with bronchiolitis obliterans due to sulfur mustard in bronchiolitis obliterans syndrome (BOS) classes 1 and 2, randomly entered Group 1 ($n = 72$, NAC) and Group 2 ($n = 72$, placebo). Dyspnea, wake-up dyspnea, cough, and sputum were measured after 4 months. Spirometric findings were measured at the beginning of the trial, 2 months after and after 4 months of prescription of 1800 mg/day in three doses of NAC or placebo.
Results: Dyspnea, cough, sputum, and wake-up dyspnea improved after 4 months of NAC compared to the control group. After 4 months, spirometric components were significantly improved in NAC group compared to placebo group.
Conclusion: Fourth months administration of NAC (1800 mg daily) can improve clinical conditions and spirometric findings in mustard exposed in BOS class 1 or 2.

Introduction

Nowadays there are more than 40,000 people suffering from pulmonary lesions due to mustard gas in Iran.[1] There is not a common consensus about the pathophysiological basis of chronic lesions of mustard gas[2,3]; but there is a huge body of

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results showing that bronchiolitis obliterans could be mentioned as basis.[4–7] So a progressive process could be assumed at the heart of its pathology. Some studies showed that mustard induced mustard lesions, like some other diseases are a neutrophil and/or lymphocyte dominant disorder (e.g. neutrophil dominated inflammatory disease).[8] Among the theories trying to explain these disorders, one suggests that neutrophils and lymphocytes secret proteases which themselves produce oxygen species.[8]

In order to treat patients with mustard induced pulmonary disorders, they received bronchodilators, corticosteroids, immunosuppressive, antibiotics, mucolytics, long-term oxygen therapy and physiotherapy.[1] These treatments have known side effects. For example, long-term administration of corticosteroids could suppress adrenal which in turn causes diabetes mellitus, osteoporosis, sodium retention, and many other disorders.[9] In parallel, these patients do not respond well to bronchodilators. So it is reasonable to look for new drugs and protocols in order to substitute the old treatments with new ones.

By considering the roles of oxygen species and free radicals in the pathophysiology of mustard induced pulmonary lesions, it is useful to consider antioxidant drugs in treating these patients.[8] *N*-acetyl cysteine (NAC) is categorized in mucolytic drugs but it also has antioxidant effects. It is used in patients with COPD and asthma to reduce signs and symptoms and improves pulmonary function tests (PFTs) and quality of life.[10,11] It also has good therapeutic effects in fibrozing alveolitis.[12] Kasielski and Nowak[13] showed that administering NAC, 600 mg daily for 12 months in patients with COPD, reduced oxidative stress. There are also some other results in the same line.[14,15]

NAC can reduce the oxidative burst of polymorphonuclear (PMN) cells in COPD patients.[16] It is also proposed that it could protect pulmonary cells from inhaled oxidants and also cytokine made ones.[17] NAC could have protective effects on peripheral granulocytes in COPD patients too.[18] It is shown that NAC reduces the number of neutrophils in mice which were exposed to sulfur mustard and developed lung injuries.[19]

So NAC could be a good alternative treatment in patients suffering pulmonary disorders due to mustard gas. NAC could be helpful with its antioxidant and also antimucolytic activities. In this trial, we tried to evaluate the therapeutic effects of NAC on late sequels of mustard induced pulmonary bronchiolitis obliterans.

Methods

In order to test the effects of NAC on pulmonary disorders due to mustard gas, we ran a placebo controlled, double blind, randomized clinical trial. Participants were among patients who suffer from pulmonary disorders due to sulfur mustard exposure. They were all from Sardasht, a city in the west of Iran; they were exposed to a single high dose of sulfur mustard in the Iran–Iraq conflict in 1988. So administering the NAC or placebo was about 18 years after their exposure. Inclusion criteria were as follows: documented exposure to sulfur mustard; documented diagnosis of chronic pulmonary lesions due to mustard; not being in a life threatening situation; not smoking cigarettes or any kind of drugs; and not having pneumonia and/or acute bronchitis. Exclusion criteria were: occurrence of any severe side effects of NAC; using any kind of antioxidant drug; history of resection of one or more lobes of lung; deterioration in clinical conditions of patients during the course of the study; and using less than 80% of the medication.

Before the trial, high resolution computed tomography (HRCT) was taken from participants. Chest HRCT scans were taken with high speed advantage scanner (General Electric Medical System, Milwaukee, Wisconsin). It consisted of five 1.0 mm collimation images obtained during deep inspiration and full expiration, while patients were in supine position. All chest HRCT scans were reviewed by a radiologist familiar with BO cases. The expiratory images were assessed for the presence of air trapping and lobar distribution of it defined as alteration of normal anterior–posterior lobar attenuation gradients and/or lack of homogenous increase in pulmonary attenuation resulting in persistent areas of decreased attenuation. The presence of air trapping was quantified and was considered to be an indication of BO only if it exceeded 25% of the cross-sectional areas of an affected lung, in at least one scanned level.[7] Participants were randomly allocated to placebo or NAC group using random number table. So we had two groups: NAC and placebo groups. We enrolled 144 patients in this study. Each group contained 72 patients.

All patients received NAC or placebo for 4 months. They received 1800 mg of NAC or placebo per day in three doses. Patients were not allowed to use any other treatments during the course of the trial expect for inhalatory Salmetrol (50 μg, twice a day) and Flixotide (250 μg, twice a day). Drugs were delivered to patients every 14 days, and their using the medication was controlled by weekly phone contacts. Patients were free to leave the study whenever they wished to. Written informed contests were obtained from all patients before the trial. The trial was approved of by the ethical committee of research center of chemical injuries.

All patients were visited by a general practitioner before and after the trial in order for the clinical condition of each patient to be assessed with especial emphasis on dyspnea, wake-up dyspnea, cough, having sputum, and hemoptysis. These signs and symptoms except for sputum and hemoptysis were quantified by a scale in which 1 denoted "no problem" and 5 or 4 denoted "the worst condition" (4 for wake up dyspnea and 5 for dyspnea and cough) which is depicted in detail in Table 1. Sputum and hemoptysis were reported as having sputum or hemoptysis or not. So we were able to analyze the changes in clinical conditions in the course of the trial in each placebo and NAC group in addition to analyzing the differences between these two groups.

All participants underwent spirometry before the trial, in the middle of the course of the trial (e.g. in month 2) and just at the finishing of the trial (e.g. the fourth month after the beginning of the trial). So we were able to analyze the changes in lung function in the course of the trial in each placebo and NAC group and also the differences between these two groups. In this study, we only enrolled participants who were in class 1 or 2 of bronchiolitis obliterans syndrome (BOS 1 and BOS 2).[20]

We analyzed cough, dyspnea, wake-up dyspnea, and sputum at the beginning of the trial between two groups

(χ^2 for sputum, *t*-test for others), after 4 months in NAC and placebo groups separately (McNemar for sputum, paired *t*-test for others) and between groups after 4 months (*t*-test). We also analyzed pulmonary function test; we analyzed forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and FEV_1/FVC during the course of the trial in each group separately and also between groups during the course of trial (repeated measure ANOVA).

Result

After 4 months of treatment, 68 patients remained in placebo group and 70 in NAC group. People who left the study were excluded from all analyses. Age did not differ significantly between two groups (mean = 45.93 (S.D. = 10.09) years old in NAC, 47.19 (11.26) years old in placebo, $p = 0.48$, *t*-test). The number of men and women in each group did not differ also (67% (46) men in placebo group; 70% (49) men in NAC group, $p = 0.85$ χ^2). Clinical characteristics of both NAC and placebo groups at the beginning of the trial are shown in Table 2. We did not analyze hemoptysis because very few patients showed this symptom at the beginning of the trial.

Table 1 Definition of scales which was used to quantify cough, dyspnea and wake-up dyspnea.

Cough	
1	I did not have cough
2	I have cough, but it is not a serious problem
3	I have cough, sometimes disturbs my work
4	I have disturbing cough, usually disturbs my work
5	I have disturbing cough, always disturbs my work
Dyspnea	
1	There is no dyspnea
2	Dyspnea exists, only in extraordinary exercises
3	Dyspnea exists in ordinary exercise
4	Dyspnea exists in mild exercise
5	Dyspnea exists in rest
Wake-up dyspnea	
1	I have never waken up due to dyspnea
2	I have waken up less than one time per week
3	I have waken up one time per week
4	I have waken up more than two times per week

Clinical data

Cough did not differ between the two groups at the beginning of the trial (mean = 2.88 (S.D. = 0.65): placebo; 3.08 (0.76): NAC; *t*-test, *p*-value = 0.097). Both NAC (3.07 (0.76): cough before the trial; 2.08 (0.40): cough after 4 months; paired *t*-test, *p*-value <0.001) and placebo (2.87 (0.64): cough before the trial; 2.25 (0.50): cough after 4 months; paired *t*-test, *p*-value <0.001) improved cough after 4 months. In order to find out whether or not NAC was more effective than placebo on cough after 4 months, we compared "delta cough" (i.e. cough after 4 months—cough before the trial) between the two groups. NAC was more effective than placebo in treating cough (-0.98 (0.83) for NAC; -0.61 (0.86) for placebo, *t*-test, *p*-value = 0.011).

Dyspnea was slightly worse in NAC group (mean = 3.10 (S.D. = 0.67): placebo; 3.38 (0.61): NAC; *t*-test, *p*-value = 0.014). NAC (3.37 (0.61): dyspnea before the trial; 2.62 (0.57): dyspnea after 4 months; paired *t*-test, *p*-value <0.001) could improve dyspnea after 4 months but placebo did not have such an effect (3.09 (0.66): dyspnea before the trial; 2.94 (0.48): dyspnea after 4 months; paired *t*-test, *p*-value = 0.06). We computed "delta dyspnea" (i.e. dyspnea after 4 months—dyspnea before the trial) and compared it between the two groups. NAC was more effective than placebo in reducing dyspnea (-0.74 (0.71) for NAC; -0.14 (0.65) for placebo, *t*-test, *p*-value <0.001).

Wake-up dyspnea did not differ between groups (mean = 2.10 (S.D. = 0.81): placebo; 2.39 (0.97): NAC; *t*-test, *p*-value = 0.06). NAC (2.37 (0.96): wake-up dyspnea before the trial; 1.86 (0.51): wake-up dyspnea after 4 months; paired *t*-test, *p*-value <0.001) improved cough after 4 months but placebo did not show such an effect (2.07 (0.78): wake-up dyspnea before the trial; 1.88 (0.56): wake-up dyspnea after 4 months; paired *t*-test, *p*-value = 0.068).

Table 2 Clinical characteristics of both NAC and placebo groups at the beginning of the trial before drug administration.

	NAC	Placebo	Analysis
Cough*	3.08 (0.76)	2.88 (0.65)	*t*-Test, $p = 0.097$
Dyspnea*	3.18 (0.61)	3.10 (0.67)	*t*-Test, $p = 0.014$
Wake-up dyspnea*	2.39 (0.97)	2.10 (0.81)	*t*-Test, $p = 0.06$
Sputumb†	87.5% (63)	79.4% (54)	χ^2, $p = 0.1$
Hemoptysis†	5.6% (4)	2.9% (2)	χ^2, $p = 0.43$

The difference between two groups for each clinical condition was analyzed separately. The test applied and the significance level was depicted in the rightmost column.

*Data are depicted as mean (S.D.).

†Data are depicted as percent (number) of patients did have sputum or hemoptysis.

We computed delta "wake-up dyspnea" (i.e. wake-up dyspnea after 4 months—wake-up dyspnea before the trial) and compared it between the two groups. NAC was more effective than placebo in reducing wake-up dyspnea (−0.50 (0.87) for NAC; −0.19 (0.85) for placebo, *t*-test, *p*-value = 0.036).

Sputum did not differ between the two groups at the beginning of the trial when 87.5% (63) in NAC and 79.4% (54) in placebo group revealed this symptom (χ^2, *p*-value = 0.1). After 4 months, NAC reduced sputum (87.5% (63) before the trial, 55% (39) before the trial, McNemar, $p<0.001$) but placebo did not (79.4% (54) before the trial, 72% (49) after the trial, McNemar, $p = 0.64$).

Pulmonary function test (PFT) data

FEV_1

Applying repeated measure ANOVA (General Linear Model) in which the changes of both groups were compared, there were not any significant differences between these changes over the course of trial ($p = 0.372$). But FEV_1 changed significantly over time in NAC group (repeated measure ANOVA, General Linear Model, $p<0.001$), but not in the placebo group ($p = 0.164$). Considering the differences of FEV_1 in both groups at the beginning of the trial in regression model, NAC significantly improved FEV_1 over placebo after 4 months ($p<0.001$). The mean (S.D.) of FVC in both groups during the trial is depicted in Figure 1.

FVC

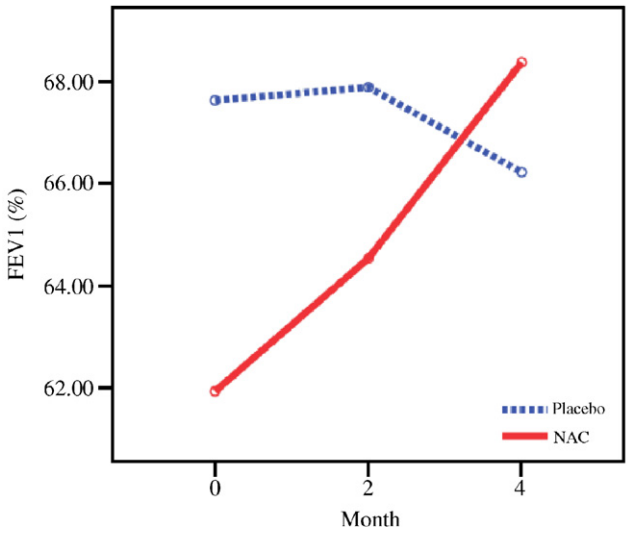
Applying repeated measure ANOVA (General Linear Model) in which the changes of both groups were compared, there were not any significant differences between these changes over the course of trial ($p = 0.610$). But FVC changed significantly over time in NAC group (repeated measure ANOVA, General Linear Model, $p = 0.004$), but not in the placebo group ($p = 0.743$). Considering the differences of FVC in both groups at the beginning of the trial in regression model, NAC significantly improved FVC over placebo after 4 months ($p = 0.044$). The mean (S.D.) of FEV_1 in both groups during the study is depicted in Figure 2.

FEV_1/FVC

Applying repeated measure ANOVA (General Linear Model) in which the changes of both groups were compared, there were not any significant differences between these changes over the course of trial ($p = 0.317$). But FEV_1/FVC changed significantly over time in NAC group (repeated measure ANOVA, General Linear Model, $p<0.001$), but not in the placebo group ($p = 0.149$). Considering the differences of FEV_1/FVC in both groups at the beginning of the trial in regression model, NAC significantly improved FEV_1/FVC over placebo after 4 months ($p<0.001$). The mean (S.D.) of FEV_1/FVC in both groups during the trial is depicted in Figure 3.

Discussion

In our study, we found out that NAC could improve not only clinical signs and symptoms of patients with mustard induced bronchiolitis obliterans, but also parameters of pulmonary function test. These findings are more interesting when we consider the time of the exposure to sulfur mustard and the time of the administration of NAC, i.e. a gap of 18 years and only a treatment of 4-month's time.



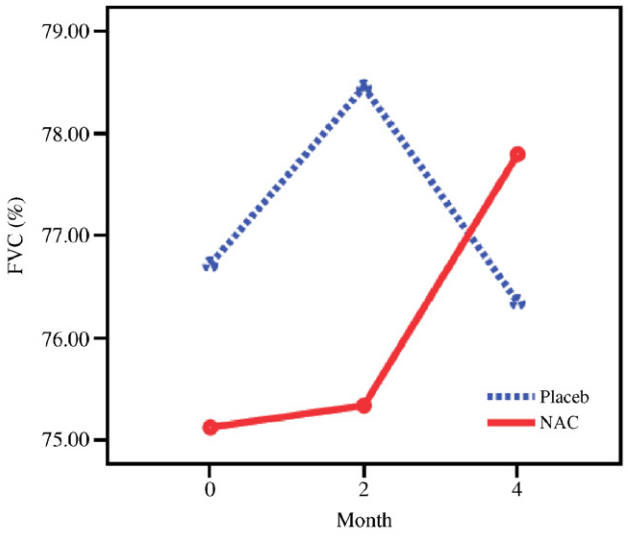
	FEV1 (0)	FEV1 (2)	FEV1 (4)
NAC	61.93 (14.77)	64.53 (16.52)	68.37 (16.72)
Placebo	67.62 (16.24)	67.88 (14.23)	66.22 (16.11)

Figure 1 The upper part represented the mean of FEV_1 in NAC group (red, continues line) and placebo group (blue, dashed line) at the beginning (month 0), after 2 months of the beginning of the trial (month 2) and after 4 months of the beginning of the trial (month 4). The table in the lower part reports the mean (S.D.) of FEV_1 in both groups over time.

Previous studies showed that NAC could be effective in the treatment of and controlling clinical conditions in COPD patients by its antioxidative properties.[21–23] It could reduce bronchial infections[24] and exacerbations[25] in these patients too. It seems that NAC interacts with inflammatory processes underlying the pathophysiology of COPD.[26–28]

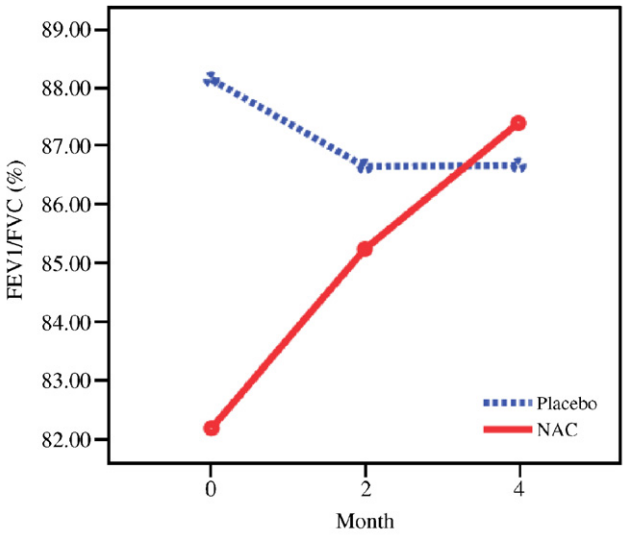
We administered NAC or placebo in combination with Flixotide and Salmetrol. Because both groups received Flixotide and Salmetrol, we could not rule out the possibility of the synergistic effects of NAC and these medications. We suggest that this possibility should be slim because previous results of our clinic showed that Flixotide and Salmetrol had improving effects on only 27% of the studied population of patients with the same conditions as this study.[29] So, planning a clinical trial with NAC administration without any other types of medications would clear this ambiguity.

Our data showed that NAC is also effective in treating bronchiolitis caused by mustard exposure. NAC could produce effects by preventing the release of many inflammatory mediators in different pathological conditions.[10] It could prevent bronchiolitis in mice which were exposed to cigarette smoke.[30] NAC could reduce the level of TNF-α in lung transplanted persons.[31] It also reduces the secretion of many inflammatory modulators (e.g. Ref. 32). Just the same, by acting on bronchial smooth muscles, it could



	FVC (0)	FVC (2)	FVC (4)
NAC	75.12 (11.92)	75.34 (13.04)	77.79 (13.08)
Placebo	76.71 (13.89)	78.44 (13.57)	76.33 (15.19)

Figure 2 The upper part represented the mean of FVC in NAC group (red, continues line) and placebo group (blue, dashed line) at the beginning (month 0), after 2 months of the beginning of the trial (month 2) and after 4 months of the beginning of the trial (month 4). The table in the lower part reports the mean (S.D.) of FVC in both groups over time.



	FEV1/FVC(0)	FEV1/FVC(2)	FEV1/FVC(4)
NAC	82.20 (14.63)	85.25 (15.14)	87.41 (13.91)
Placebo	88.15 (13.54)	86.66 (12.19)	86.68 (12.60)

Figure 3 The upper part represented the mean of FEV_1/FVC in NAC group (red, continues line) and placebo group (blue, dashed line) at the beginning (month 0), after 2 months of the beginning of the trial (month 2) and after 4 months of the beginning of the trial (month 4). The table in the lower part reports the mean (S.D.) of FEV_1/FVC in both groups over time.

prevent thickening of respiratory airways and bronchial smooth muscle hypertrophy.[33]

So NAC could reduce the inflammation in respiratory system by characteristics mentioned above and our results are also in line with the effectiveness of NAC in treating bronchiolitis due to sulfur mustard exposure. It is concordant with the evidence that showed the effectiveness of antioxidants in preventing sulfur mustard induced oxidative stress.[34,35] It is also in harmony with the outcomes of the experimental studies. NAC could protect bronchial epithelial cells against sulfur mustard in vitro.[17,36] NAC could also treat acute lung injuries induced by mustard gas in rat model.[37] So NAC not only can be used in treating bronchitis, but also in treating *Bronchiolitis*; not only in preventing mustard induced oxidative stress, but also in treating mustard induced pulmonary lesions.

Conflict of interest

Authors had conflict of interest because drugs were provided by Zambon Co. Because all the stages of this study were done in a double blind fashion, the conflict of interest did not affect the results.

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