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Research Article

Pharmacodynamic Effect of N-Acetylcysteine as Adjunctive Therapy in Mild Systemic Lupus Erythematosus Patients

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Abstract

***Purpose**: To evaluate the pharmacodynamic effect of N-acetylcysteine (NAC) in mild systemic lupus erythematosus (SLE) patients in order to implement NAC as adjunctive therapy in SLE population.*
***Methods:** Forty mild SLE patients were randomly allocated to receive 1800 mg of NAC with SLE standard therapy or receive standard therapy alone. Follow up was performed at 2 weeks, then every month up to 6 months. Glutathione GSH and malondialdehyde (MDA), a lipid peroxidation product in plasma, were measured at each visit; clinical symptoms were also evaluated.*
***Results:** Supplementation with 1800 mg of NAC did not significantly ($p > 0.05$) affect GSH level; however, MDA level was significantly ($p < 0.05$) decreased and the number of patients who could tapper prednisolone dosage was higher in the NAC group than in the control group.*
***Conclusion**: Administration of NAC may be beneficial in mild SLE patients in terms of decreasing lipid peroxidation. However, this conclusion does not apply to the effect of NAC at different dosages for moderate and severe SLE. Further studies are required in this regard*

***Keywords:** Pharmacodynamics, N-Acetylcysteine, Systemic lupus erythematosus, Adjunctive therapy.*

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INTRODUCTION

N-acetylcysteine (NAC) is an old nutrient which is indicated as a mucolytic agent and antidote for paracetamol intoxication. It is a glutahione (GSH) precursor and is deacetylated to cysteine (Cys), amino acid which is a substrate of GSH biosynthesis enzyme. GSH is an important biomolecule which has high antioxidant activity[1]. There have been studies of its role in many diseases such as cardiovascular diseases, diabetes, HIV infection and cancer [1-3]. GSH may be a marker of longevity [3-5].

In systemic lupus erythematosus (SLE), an imbalance of oxidative status has been found in some studies. Additionally, enhancement of lipid peroxidation and deficiency of antioxidant status were observed [6-12]. In deficiency of antioxidative status in SLE patients, supplementation with a glutathione precursor has been used as a strategy for the reduction of active symptoms in SLE patients. Therefore, the aim of this study was to evaluate the pharmacodynamic effect and safety profile of NAC in mild SLE patients on standard therapy.

PATIENTS AND METHODS

Patients

Following approved by the Ethics Committee of Ramathibodi [13], 40 SLE patients were randomly assigned to 2 groups (control and NAC administration) and an attempt was made to match them with age, sex and medication. The demographic data for these 2 groups was recorded.

The inclusion criteria were (a) males or females aged > 18 years who were diagnosed with SLE according to the American College of Rheumatology (ACR) criteria and in whom the condition was mild according to the definition; (b) patients who received standard treatment for mild SLE; and (c) patients who were willing to be included in the study and signed the informed consent form. Patients were excluded if they (a) were poorly compliant with drug regimens by interview or any evidence (such as unused medications); (b) were pregnant or lactating; (c) had hypersensitivity or intolerance to NAC; (d) had infectious or liver diseases; and (e) were considered by their physician as unsuitable to be included in this study.

Drug administration

One group received standard therapy and another group received 600 mg of N-acetylcystiene [NAC] thrice daily. The effectiveness of this dosage in avelolitis patients who received co-administration with maintenance immunosuppressives is well known.

Study monitoring and pharmacodynamic evaluation

Five milliliters of blood samples was collected at each visit. Blood samples were drawn and immediately chilled in iced box. Plasma was separated by centrifugation at 2 °C and 3000 rpm for 10 min. These specimens were then kept at -20°C and analyzed within 2 weeks.

Glutathione analysis was performed using glutathione assay kit (Bioassay system Inc®, California, USA). Malondialdehyde (MDA), a lipid peroxidation product, was measured according to the method of Yagi [14], using a microplate reader (Victor^2 Pergin Almer Inc®, CA, USA) as a detector.

The demographic data and baseline characteristics of both groups were recorded. The following pharmacodynamic parameters at baseline, 2 weeks, then every month up to 6 months were measured: plasma glutathione (GSH), plasma MDA. In addition, the number of patients who could tapper prednisolone were recorded; follow-up was set at 2 weeks, then monthly up to 6 months. SLE activity index SLEDAI scores at baseline and 6 months were also recorded for evaluation of clinical outcomes. The clinical outcomes and safety profile of NAC with standard therapy were also evaluated.

Data analysis

Both dependent and independent Student t-tests were used to test differences in GSH and MDA concentration of the 2 groups at base line and 6 months in order to determine the efficacy of NAC. Chi-square test was used to compare the number of SLE patients in the 2 groups who could tapper prednisolone. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The demographic, medication and laboratory data for the SLE patients are listed in Table 1. Patients in the 2 groups had almost similar characteristics, except platelet and ALT.

The effect of NAC on GSH, MDA and SLEDAI score are shown in Table 2. The analytical methods for GSH and MDA were validated and they gave coefficient of variation (CV) of < 10 % for intra-day and inter-day precision, and recovery was > 90 %. Correlation coefficient was 0.99.

There was a significant difference ($p < 0.05$) in the plasma GSH levels of the 2 groups but the difference was not significant ($p < 0.05$) in the case of MDA levels. In addition, there was a statistical difference in proportion of SLE patients who could tapper prednisolone. All the patients in the NAC group could tapper prednisolone while only 13 patients in the control group could reduce prednisolone dose. Chi-square test data are shown in Tables 3 and 4, respectively. Both 2 groups could significantly decrease in prednisolone dose based on comparison of prednisolone dose at baseline and 6 months.

However, when subgroup analysis according to SLEDAI score was performed, it found that only NAC groups showed a significant decrease in prednisolone dosing at 6 months, as indicated in Table 5. However, this occurred was only among those in NAC group whose SLEDAI score was < 4 and ≥ 4, as shown in Table 5 ($p < 0.005$**)**.

Table 1: Dermographics, medication and laboratory characteristic of SLE patients

Characteristics	Control (n=20)	NAC group (n=20)	*p*-value
Age (year, mean ± SD)	36 ± 8.5	35 ± 0.1	0.72
Sex	Female	Female	1.00
Duration of SLE (year, mean ± SD)	10.0± 6.9	9.0± 6.4	0.604
Drugs			
Predinsolone	18	17	0.500
Hydroxychloroquine	13	17	0.273
Azathioprine	7	7	0.629
NSAIDs	4	4	0.653
Prednisolone +hydroxychloroquine	11	15	1.00
Prednisolone +azathioprine	7	5	1.00
Prednisolone + hydroxychloroquine +Azathioprine	4	3	1.00
Laboratory data			
Hemoglobin (mg/dL, mean ± SD)	12.0 ± 0.9	12.3 ± 1.5	0.426
Hematocrit (mg/dL, mean ± SD)	35.5± 3.0	36.5± 0.4	0.437
SLEDAI score	3	2	0.426
WBC (cell/m^3 , mean ± SD)	5776± 2156	6713± 3957	0.269
Plt (cell/m^3 , mean ± SD)	248055 ± 92203	314900± 81721	0.020*
ESR (h^{-1} , mean ± SD)	34.2 ± 22.8	33.1 ± 24.9	0.851
SCr (mg/dL, mean ± SD)	0.786 ± 0.335	0.760± 0.210	0.891
AST ((IU. , mean ± SD)	36.7 ± 1.2	47.3 ± 27.6	0.347
ALT ((IU. , mean ± SD)	87.8 ± 38,4	36.6 ± 18.4	0.039*
MDA (µM/L, mean ± SEM)	0.362 ± 0.042	0.417 ± 0.050	0.479
GSH (µM/L, mean ± SEM)	574.6 ± 84.0	673.5 ± 70.8	0.401

**Significant difference at p < 0.05; Plt = platelet. ESR = erythrocyte sedimentation rate, Scr = serum creatinine, AST = aspartate trasferase, ALT = alanine trasferase, MDA = malondialdehyde, GSH=glutathione*

Table 2: Effect of N-acetylcysteine on oxidative status parameters (GSH and MDA) and SLE activity Index (SLEDAI) in 6 months

Parameter	Group	Baseline (mean± SEM)	6 months (mean± SEM)	*p* value
MDA (µM)	Control	0.362 ± 0.042	0.371 ± 0.042	0.875
	NAC	0.417 ± 0.050	0.291 ± 0.033	0.023*
GSH (µM)	Control	575 ± 84	839 ± 162	0.174
	NAC	673 ± 71	702 ± 133	0.702
SLEDAI	Control	2.65 ± 1.46	1.50± 1.47	0.003*
	NAC	2.15± 1.53	0.95± 1.28	0.001*

** Statistically significantdifference at p < 0.05*

Table 3: Number of SLE patients who could tapper prednisolone

Prednislone tapper	Group*		Total
	Control	*NAC*	
No	7	0	7
Yes	13	20	33
Total	***20***	***20***	***40***

** p = 0.008*

Table 4: Chi-square test of prednisolone tappering in control versus NAC group

Test type	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.485	1	.004		
Continuity Correction	6.234	1	.013		
Likelihood Ratio	11.200	1	.001		
Fisher's Exact Test				.008*	.004*
Linear-by-Linear Association	8.273	1	.004		
No. of valid cases	40				

*Note: Data computed only for a 2x2 table; two cells (50 %) showed expected count < 5, the minimum expected count being 3.50; * Statistically significant*

Table 5: Effect of NAC on prednisolone dose in 6 months

Parameter (mean ± SEM)	Group	N	Baseline	6 months	*p* value
Prednisolone dose (mg) for all subjects in both groups	Control	20	130.8 ± 31.1	53.3 ± 10.0	0.012*
	NAC	20	118.4 ± 25.5	47.5 ± 11.4	0.002*
Prednisolone dose (mg) of subjects who had SLEDAI ≥ 4 in both group	Control	9	172.2 ± 68.4	68.8 ± 29.4	0.124
	NAC	6	150.6 ± 70.8	59.2 ± 37.2	0.014*
Prednisolone dose (mg) of subjects who had SLEDAI < 4 in both groups	Control	11	141.6 ± 42.7	51.3 ± 15.5	0.274
	NAC	14	97.50 ± 17.57	38.39 ± 10.31	0.0001*

**Significant*

Clinical outcomes and adverse effects of NAC

Based on random interview of patients who received NAC, four patients said they felt better, fresher, and had better skin appearance. In addition, an interview with one physician who took care of the patients who received NAC showed that the clinical outcomes of the patients improved, as

indicated,by laboratory test results, improved clinical symptoms such as decreased hair loss, as well as absence of new rash, fatigue and joint pain. However, during the study, 4 patients were withdrawn and and substituted with SLE patients in the NAC group. Among them, one patient experienced shortness of breath; another developed a rash and two experienced headache. These symptoms, however, disappeared when the drug was discontinued.

DISCUSSION

Based on the results obtained, administration of 1800 mg NAC as adjunctive therapy did not produce a significant change in GSH level, while the MDA level of NAC group was significantly lower than in the control group during the six months of therapy. This result was similar to the phase I study on the effect of NAC as a chemopreventive in patients who suffered from malignancy [15]. In that study, there was no correlation between NAC concentration and change in GSH in peripheral blood lymphocyte; furthermore, the effect of NAC as a glutathione precursor was prolonged only for a short time and this positive effect occurred only in some patients [16]. This may be due to the pro-oxidant effect of NAC which was found the in vitro study [17]. High doses of NAC might have caused the increased formation of free radicals and thus resulted in lower GSH level than in the control group. Significant anti-oxidant effect [18-20] was also found by lowering MDA levels during the 6 months of therapy.

Consideration of the mechanism of action of NAC which may provide an insight into the understanding of the results obtained. Although deacetylation of NAC releases cysteine which is the primary agent for glutathione synthesis, the synthetic process of GSH does not only depend on cysteine. It also requires two other amino acids - glycine and glutamine. Therefore, increased GSH levels resulting from NAC therapy sometimes does not rise sharply if glycine and glutamine levels are inadequate. In contrast, MDA synthesis originates from lipid peroxidation and is not dependent on other agents. Therefore, lipid peroxidation effect, which is inhibited by NAC, increased markedly, leading to a significant decrease in plasma MDA level. Polymorphism of glutathione synthase enzyme and SLE has also been established [21-23].

All the patients in NAC group could tapper prednisolone while only 13 patients in the control group were able to do so. in this regard, the difference between the two groups was statistically significant different ($p = 0.008$).

Limitations of study

This study had a couple of limitations, the first being that only one dose of NAC was administered, and therefore, the results are based on administration at one dose level, i.e., 1800 mg. Further studies are required to determine the outcome at other dose levels of NAC. Another limitation has to do with the level of severity of the SLE patients since only mild SLE patients were recruited. Expansion of the study to moderate or severe SLE patients could have possibly resulted in a different outcome.

CONCLUSION

Administration of 1800 mg NAC may be beneficial to SLE patients who who suffer minor symptoms and are able to tapper prednisolone which may cause many side effect. Based on these results, it would be necessary to study the effect of N-acetylcystiene in moderate and severe SLE cases as this would facilitate the implementation of NAC therapy for moderate and severe SLE patients.

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