ผลทางเภสัชพลศาสตร์ของการให้ N-acetylcysteine เป็นยาร่วมในผู้ป่วย systemic lupus erythematosus ชนิดไม่รุนแรง

น.ส. กรัณฑ์รัตน์ ทิวถนอม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชาการบริบาลทางเภสัชกรรม ภาควิชาเภสัชกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

THE PHARMACODYNAMIC EFFECT OF N-ACETYLCYSTEINE AS ADJUNCTIVE THERAPY IN MILD SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENTS

MISS KARUNRAT TEWTHANOM

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Pharmaceutical care
Faculty of Pharmaceutical Sciences Chulalongkorn University
Academic year 2007

Thesis Title	THE PHARMACODYNAMIC EFFECT OF N-ACETYLCYSTEINE AS ADJUNCTIVE THERAPY IN MILD SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENTS
By	Karunrat Tewthanom
Filed of study	Pharmaceutical care
Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
Thesis Co-advisor	Professor Suchela Janwityanujit, MD.
Thesis Co-advisor	Assistant Professor Kitti Totemchokchyakarn, MD.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

..... Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Associate Professor Pintip Pongpech, Ph.D.)

..... Thesis Advisor

(Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.)

..... Thesis Co-advisor

(Professor Suchela Janwityanujit, MD.)

..... Thesis Co-advisor

(Assistant Professor Kitti TotemChockchyakarn, MD.)

..... Member

(Associate Professor Pornanong Aramwit, Pharm. D., Ph.D.)

..... Member

(Assistant Professor Sunee Tahchaarpornkul, Ph.D.)

..... Member

(Miss Walapa Tatong, Ph.D.)

กรัณฑ์รัตน์ ทิวถนอม:ผลทางเภสัชพลศาสตร์ของการให้ N-acetylcysteine เป็นยาร่วมในผู้ป่วย systemic lupus erythematosus ชนิดไม่รุนแรง (THE PHARMACODYNAMIC EFFECT OF N-ACETYLCYSTEINE AS ADJUNCTIVE THERAPY IN MILD SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENTS) อ. ที่ปรึกษา : เภสัชกรหญิง รศ. ดร. ดวงจิตต์ พนมวัน ณ. อยุธยา, อ.ที่ปรึกษาร่วม : ศ. พญ. สุชีลา จันทร-วิทยานุชิต, ผศ. นพ. กิตติ โตเต็มโชคชัยการ 111 หน้า.

ในการศึกษานี้ ได้นำ N-acetylcysteine (NAC) ซึ่งมีฤทธิ์ในการเป็นสารต้านอนุมูลอิสระสูงมาเป็นยาร่วมในการรักษาผู้ป่วย systemic lupus erythematosus (SLE) เพื่อศึกษาว่าจะสามารถแก้ไขผลของความไม่สมดุลย์ของภาวะออกซิเดชั่นหรือไม่ โดยแบ่งการศึกษาเป็น 3 ส่วน ส่วนแรกเป็นการศึกษาระบาดวิทยาโดยการหาความชุกของโรค SLE ในแผนกอายุรกรรม โรงพยาบาลรามาธิบดี ระหว่าง ปี 2543-2549 และศึกษารูปแบบของการให้ยาเมื่อเปรียบเทียบกับแนวทางการรักษามาตรฐาน ส่วนที่ 2 เป็นการศึกษาภาวะออกซิเดชั่นในผู้ป่วย SLE ที่มีระดับความรุนแรงต่างกัน 54 ราย (อาการรุนแรงน้อย 20 ราย ปานกลาง 20 ราย และ รุนแรงมาก 14 ราย) เปรียบเทียบกับในอาสาสมัครสุขภาพดี 20 ราย โดยการวัดระดับ กลูตาไทโอน (GSH) ในพลาสมา (GSH ซึ่งเป็นสารต้านอนุมูลอิสระธรรมชาติในพลาสมา) และระดับ มาลอนไดอัลดีไฮด์ (MDA) ซึ่งเป็นสารบ่งชี้สภาวะออกซิเดชั่นของไขมัน ส่วนที่สามศึกษาผลทางเภสัชพลศาสตร์ของ NAC ที่มีต่อการเปลี่ยนแปลงระดับ GSH และ MDA ในพลาสมาของผู้ป่วย SLE ชนิดไม่รุนแรงเทียบกับกลุ่มควบคุมที่ไม่ได้ NAC (n= 20 ต่อกลุ่ม) เป็นเวลา 6 เดือน ผลการศึกษาพบว่า ความชุกของโรค SLE ในแผนกอายุรกรรม อยู่ระหว่าง 267 – 552 ราย ต่อ ผู้ป่วย 100,000 ราย ที่เข้ารับการรักษาในแผนกอายุรกรรม รูปแบบการให้ยาที่นิยมสั่งจ่ายอิงแนวทางการรักษามาตรฐาน โดยมีเพรดนิโซโลนร่วมด้วยเป็นส่วนใหญ่ และมักจะใช้รูปแบบยาที่ประกอบด้วยยามากกว่า 1 ขนาน ยาหลักที่ใช้ได้แก่ เพรดนิโซโลน คลอโรควิน อะซาไทโอ-ปริน และไฮดรอกซี่คลอโรควิน พบความสัมพันธ์อย่างมีนัยสำคัญระหว่างระยะเวลาที่เป็นโรคและสัดส่วน (ร้อยละ) ของผู้ป่วยที่ได้รับรูปแบบยาที่มีอะซาไทโอปริน ($p<0.05$) การศึกษาที่เกี่ยวข้องกับการวัดภาวะออกซิเดชั่นพบว่ามีความสัมพันธ์ระหว่างระดับกลูตาไทโอนกับความรุนแรงของโรค (วัดโดยใช้ systemic lupus erythematosus disease activity index, SLEADI score) $p<0.05$ ขณะที่ไม่พบความสัมพันธ์ดังกล่าวเมื่อพิจารณาระดับ MDA ในพลาสมา การศึกษาในส่วนสุดท้ายพบว่า กลุ่มที่ให้ NAC ร่วมด้วยมีระดับ MDA ในพลาสมา ที่เวลา 6 เดือนลดลงต่ำกว่ากลุ่มที่ไม่ได้ NAC (0.507 ± 0.274 vs 0.354 ± 0.178 μM, $p= 0.023$) ในขณะที่ไม่พบความแตกต่างอย่างมีนัยสำคัญของ GSH ในพลาสมา นอกจากนี้พบว่ากลุ่มที่ได้รับ NAC มีอัตราส่วนของผู้ป่วยที่สามารถลดขนาดเพรดนิโซโลนลงได้มากกว่าอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) จึงสามารถสรุปได้ว่า NAC น่าจะมีแนวโน้มที่จะส่งผลต่อ MDA ในพลาสมา และมีส่วนสัมพันธ์ในการลดขนาดเพรดนิโซโลนในผู้ป่วย SLE ที่มีอาการไม่รุนแรง การขยายผลการศึกษาถึงเภสัช-พลศาสตร์ของ NAC ไปยังกลุ่มผู้ป่วย SLE ที่มีอาการรุนแรงมากขึ้นซึ่งน่าจะเห็นผลได้ชัดเจนกว่าซึ่งทำให้สามารถสรุปได้อย่างมั่นใจขึ้นว่าควรจะนำ NAC มาใช้เป็นยาร่วมในผู้ป่วย SLE หรือไม่

สาขาวิชา การบริบาลทางเภสัชกรรม ลายมือชื่อนิสิต .....
ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา .....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

4576952633 : MAJOR PHARMACEUTICAL CARE

KEY WORD : PHARMACODYNAMICS/ N-ACETYLCYSTEINE / SYSTEMIC LUPUS ERYTHEMATOSUS/ ADJUNCTIVE THERAPY

KARUNRAT TEWTHANOM: THE PHARMACODYNAMIC EFFECT OF N-ACETYLCYSTEINE AS ADJUNCTIVE THERAPY IN MILD SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENTS THESIS ADVISOR: ASSOC PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D. THESIS COADVISOR: PROF. SUCHELA JANWITYANUJIT, MD., ASIST. PROF. KITTI TOTEMCHOCKCHYAKARN, MD., 111 pp.

N-acetylcysteine (NAC), a strong free radical scavenger, was designed to use as adjunctive therapy in mild systemic lupus erythematosus (SLE) to correct an imbalance of antioxidant status which is previously reported in SLE patients. This study was divided into 3 parts. Firstly, epidemiology and drug pattern used which were determined by the prevalence of SLE in department of medicine, Ramathibodi hospital, THAILAND during 2000-2006 while the drug pattern information was recorded and was compared to standard guideline therapy. Secondly, the oxidative status was compared among 54 SLE patients with different degree of severity of the disease (mild =20, moderate=20, severe=14) and among 20 healthy volunteers; plasma glutathione (GSH; a natural antioxidant) and plasma malondialdehyde (MDA; the marker of lipid peroxidation) were measured. Thirdly, pharmacodynamic effect of NAC in term of alteration in plasma GSH and plasma MDA levels as compare to that in the control group and in mild SLE patients (n=20 in each group) for 6 months. The results indicated that the prevalence of SLE was ranged from 267 -552 per 100,000 visitors. Prednisolone containing regimens and combined-drug were frequently prescribed in SLE patients. The four main drugs; prednisolone, chloroquine, azathioprine, and hydroxychloroquine were used. The significant association between SLE duration and the proportion (percentage) of patients who received azathioprine was found. In part of oxidative stress monitoring, there was a significant correlation of plasma GSH level and SLE severity (measured by systemic lupus erythematosus disease activity index, SLEDAI score), $p<0.05$. Whereas, such correlation was not observed in termed of plasma MDA. Finally, NAC administration was beneficial in significant reducing plasma MDA level at 6 months (0.507 ± 0.274 vs 0.354 ± 0.178 μM, $p=0.023$), while plasma GSH was not shown to be significant difference. Furthermore, significant higher proportion of SLE patients who could taper prednisolone dosage was found in NAC group ($p<0.05$). It can be concluded that NAC seems to be beneficial in reducing plasma MDA level and associate in tapering prednisolone in mild SLE patients. The pharmacodynamic effects of NAC should be expand to investigated in more severe SLE patients to make a more confident conclusion on the benefit of using NAC as an adjunctive therapy in SLE patients.

Field of study Pharmaceutical care

Academic year 2007

Student's signature .....

Advisor's signature .....

Co-advisor's signature .....

Co-advisor's signature .....

ACKNOWLEDGEMENTS

I would like to express my great appreciation to my advisor , Associate Professor Duangchit Panomvana Na Ayudhya, for her invaluable suggestion meaningful guidance, warmly care and fervent encouragement throughout my study.

I am very grateful to my co-advisor, Professor Suchela Janwitayanujit, for her helpful guidance and offering an opportunity to perform this dissertation at Division of Allergy, Immunology and Rheumatology, Department of Medicine, Ramathibodi hospital, Mahidol University, Thailand.

My special thanks are extended to Associate Professor Kitti Totemchokchyakarn, my co-advisor for his helpful consultation and comments.

I wish to extend my grateful appreciation to all members at Division of Allergy, Immunology and Rheumatology, Department of Medicine, Ramathibodi hospital, for their kind co-operation during this study.

My sincere thanks are also extended to all patients and volunteers who participated in this study for their kind co-operation.

I wish to acknowledge the director of SM Pharmaceuticals company, Thailand for supporting NAC (Fluimucil®) products.

I gratefully acknowledge financial assistance from the Graduate school, Chulalongkorn University which enables me to perform this research.

I am deeply indebted to the funding from Faculty of Pharmacy, Silpakorn University which enable me to undertake this course of study.

Finally, my indescribable gratitude is dedicated to my beloved parents, sister, brother and my colleges at Silpakorn University for their enthusiasm, kindness and understanding; they are the strong inspiration for me to succeed in my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

Appendix tables

LIST OF TABLES (Cont.)

LIST OF TABLES (Cont.)

LIST OF FIGURES

LIST OF FIGURES (Cont.)

ABBREVIATIONS

Abbreviations used in the text are listed as the following

SLE : Systemic lupus erythematosus

ANA : Antinuclear antibody

ACR : American college of rheumatology

NAC : N-acetylcysteine

BL : Peripheral blood lymphocyte

e-NOS : Endothelial nitric oxide synthase

BH_4 : Tetrahydrobiotein

SOD : Superoxide dismutase

SMC : Smooth muscle cells

VCAM : Vascular cell adhesion molecule

RCIN : Radio contrast induced nephropathy

ROS : Reactive oxygen species

RNS : Reactive nitrogen species

TBARS : Thiobarbituric acid reactions

HNE : 4-hydroxy-2 nonenal

COPD : Chronic obstructive pulmonary disease

ARDS : Acute respiratory distress syndrome

IPF : Idiopathic pulmonary fibrosis

SLEDAI : Systemic lupus erythematosus disease activity index

TBA : Thiobarbituric acid

MDA : Malondialdehyde

GSH : Glutathione

Pred : Prednisolone

Cq : Chloroquine

HCq : Hydroxy chloroquine

CYP : Cyclophosphamide

Aza : Azathioprine

MMF : Mycophenolate mofetil

CHAPTER I
INTRODUCTION

Significance of problem

N-acetylcysteine (NAC) is a thiol (sulfhydryl-containing) acetylated of the amino acid L-cysteine. This drug is converted in the body into the metabolite which is capable of enhancing GSH synthesis, promoting detoxification, and acting as a strong free radical scavenger. [3, 4] Until recently, NAC was only used as an mucolytic agent in respiratory diseases. However, current clinical applications of NAC in various inflammatory diseases have been increasing because of its antioxidant activity with low side effect and the important of oxidative stress in various inflammatory diseases. In addition, more molecular mechanisms of NAC have still been studied. It was found that NAC could also prevent apoptosis and promote cell survival. [5, 6]

Since, there is an imbalance of oxidant/antioxidant status in SLE patients. Moreover, the oxidative DNA damage and abnormal apoptosis were reported in such patients.[7, 8] Supported data from animal study showed that NAC has antioxidant activity and could suppress mortality in the female NZBxNZWF1 mouse model of SLE.[9] However, there is lack of data from human to confirm the benefit of NAC in alleviate lupus symptoms including the basis of its pharmacodynamics (PD) in this group of patients. Therefore, further researches are needed to confirm these topics .

Base on the available data, NAC may be useful as an adjunctive therapy in SLE patients. At present, NAC is more popularly used as an antidote of paracetamol intoxication and there is gradually increase of evidence based on using NAC to reduce toxic effect of radiographic contrast agents.[3] It is, therefore, challenging to conduct researches on the effect of NAC and its pharmacodynamics in mild SLE patients in order to implement the new indication.

Research Objectives

This study is aim to

1. evaluate the pharmacodynamic of NAC in mild SLE patients
2. evaluate the association between oxidative stress status and severity of SLE
3. evaluate the effect of administration of NAC as adjunctive therapy in mild SLE patients.

Expected Benefits

1. The association between oxidative stress and severity of SLE and efficacy of NAC will be identified.
2. The efficacy and adverse effects of NAC in mild SLE patients will be determined.
3. The relationship of NAC pharmacodynamics and oxidative stress if any will be revealed.

CHAPTER II

LITERATURE REVIEW

1. Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus is an autoimmune disease with variable characteristics of symptoms. This disease affects joints, skin, heart, lungs, CNS, kidneys, and hematopoietic system as illustrated in Table 1.[10]

Table 1. Clinical features of Systemic Lupus Erythematosus

Affected organ system	Percentage of patients	Signs and symptoms
Constitutional	50-100	Fatigue, fever (in the absence of infection), weight loss
Skin	73	Butterfly rash, photosensitivity rash, mucous membrane lesion, alopecia, Raynaud's phenomenon, pupura, urticaria, vasculitis
Musculoskeletal	62-67	Arthritis, arthralgia, myositis
Renal	16-38	Hematuria, proteinuria, cellular cast, nephrotic syndrome
Hematologic	36	Anemia, thrombocytopenia, leukopenia
Reticuloendothelial	7-23	Lymphadenopathy, splenomegaly, hematomegaly
Neuropsychiatric	12-21	Psychosis, seizures, organic brain syndrome, transverse myelitis, cranial neuropathies, peripheral neuropathies
Gastrointestinal	18	Nausea, vomiting, abdominal pain
Cardiac	15	Pericarditis, endocarditis, myocarditis
Pulmonary	2-12	Pleuisy, pulmonary hypertension, pulmonary parenchyma disease

1.1 Epidemiology

Only one population-based screening study of SLE reported a prevalence of 200 cases per 100,000 women (18-65 years of age) in England.[10] An estimation of overall U.S. prevalence of definite SLE plus incomplete SLE (disease meeting only some diagnosis requirement for SLE) was 40 to 50 cases per 100,000 persons.[11] In the United States , SLE is reported to be more common in white women.[11] One U.S. retrospective study on medical records showed that the disease was diagnosed 23 times more often in black women, than in white men.[11] The prevalence of the disease is also higher in Hispanic and Asian Americans.[12] The report from expert in 10th Asia–Pacific League of Associations for Rheumatology (APLAR) Congress revealed that the prevalence of SLE varies from 70.41/100,000 in China to 15/100,000 in New Zealand.[13] In addition, a familial predisposition to SLE has been identified.[12, 14-16] From the pathological studies.[17] showed that organ damage was found in SLE and such damage progressed over time. A cohort study showed that within seven years of diagnosis, 61% of patients developed clinically detectable organ damage.[17] Most commonly affected organ systems were neuropsychiatric (20.5%), musculoskeletal (18.5%), and renal (15.5%) , respectively.[17]

Remission of SLE is not uncommon but often is punctuated by flares. In a six-year prospective cohort study, disease flares occurred at a rate of 0.2 per year per patient.[10]

Mortality rates for SLE are particularly high in children.[10] In a retrospective study of Brazilian children, overall mortality during 16 year of follow up was 24%.[10] Death occurred because of infection (58%) , central nervous system disease (36%), and renal disease (7%).[10]

In Thailand, the most complication of SLE was similar to previous reports. The study at Ramathibodi hospital showed that infection occurred in 25.5% of patients.[18] while the study at King Chulalongkorn Memorial hospital showed that lower respiratory tract infection was the most commonly found in these patients (24.6%) followed by infections of the urinary tract (15.7%), skin (15.7%), septicemia (13.6%) and the musculoskeletal system (11.5%).[19] The most common pathogens were *Salmonella spp* (12.6%), while the rest were *Escherichiae coli* (9.9%) and *Mycobacterium tuberculosis* (8.4%), respectively.[19] In addition, study at Chiangmai University hospital showed that infection contributed to the death of about 51.9% SLE patients.[20] This information represents the importance for healthcare providers to concern about the management of SLE patients and also encourages establishing the researches in this field. Study about epidemiology of this disease have been performed in several countries which is presented in Table 2.

Table 2 Epidemiologic information [21]

Country	Incidence (per 100,000 per year)	Prevalence (per 100,000 per year)
United States		
All races	5.1	52.2
White	1.4	7.4
Black	4.5	19.5
Puerto-Rican	2.2	18.0
Canada		
White	1.6	20.6
First Nation	4.7	42.3
Finland	NA	28.0
France	5.0	40.0
Ireland	3.3	35.9
Italy	NA	71.0
Northern Ireland	NA	25.4
Spain		
All races	NA	91.0
White	2.2	34.1
Sweden	4.7	42.0
United Kingdom		
All races	3.8	26.2
White	3.0	20.5
Asian	10.0	47.8
China	NA	92.9
Afro-Caribbean	21.9	159.4
Australia		
White	NA	19.3
Aboriginal	11.0	63.3
Japan	2.9	28.4
Martigue	4.7	64.2

NA= Not applicable

1.2 Diagnosis

The diagnosis of SLE is based on clinical and laboratory criteria. The criteria set developed by the American College of Rheumatology (ACR) is most widely used as shown at Table 3.[10] An algorithm for the diagnosis of the disease is provided in Figure 1.[10]

1.3 Pathogenesis and oxidative stress theory [21]

The etiology of lupus remains unclear, although the apoptosis can explain how the immune system recognise predomonantly intracellular antigens. It can assume that auto antigens are released by both necrotic and apoptotic cells. Disorder or abnormal clearing of apoptotic cell lead to defect of macrophage, T cell and B cell and then driving to auto immune process. Cytokines may also involve with SLE. [21-24] Over expression of type 1 interferon was found. [24] Association of interferon regularly factor 5 (IRF 5) with SLE was also markable as an important genetic risk factor for the disease.

Disorder of signal transduction was suspected as an important cause in pathogenesis of SLE. Impaired translocation of nuclear factor ΚB, p65, decrease expression of T cell receptor, δ chain and proteine kinase C, decrease protein kinase C dependent protein phosphorylation and decrease production of interleukin-2 have been described in patients with SLE [23, 25]

Evidence of complex genetic contribution has been reported, with are increased incidence in families in which one or more members already has the disease [16]

Although the pathogenesis of SLE is likely to be multifactorial, the inflammatory nature of SLE represent that a state of oxidative stress may exist in this disease, which contribute to immune cell dysfunction, autoantigen production and autoantibody reactivity. Seyithan et al.[26] found the relationship between the level of oxidative stress and the severity of the clinical condition in SLE patients.[26] Disease activity index correlated positively with serum

malondialdehyde ($r = -0.47$, $p < 0.05$). Such relationship suggests a cause and effect; the higher the oxidative stress, the worse clinical condition of the SLE patient. The author suggested that determination about effect of supplementation of antioxidants in the treatment of those conditions should be warranted.

1.4 Therapeutic strategies

The therapeutic strategies of SLE depends on disease severity, the standard therapy is briefly summarized as following [22]

1. administration of the lowest dose of steroids that are necessary to control symptoms which occur via inflammatory process
2. administration of steroid sparing agents such as Non Steroidal Anti-inflammatory Drugs (NSAIDs), antimalarials and other immunosuppressives (azathioprine, cyclophosphamide)
3. coadministration of antacid with NSAIDs
4. supplementation of calcium and vitamin D
5. treatment of opportunistic infection which may be occurred
6. coadministration of antihypertensives or lipid lowering agents

The new treatments of this disease which can control active symptoms are summarized in Table 4.

Table 3. American College of Rheumatology classification criteria for SLE. [10]

The diagnosis of SLE requires the presence of four or more of the following 11 criteria, serially or simultaneously, during any period of observation

1. Malar rash: fixed erythema, flat or raised, over the malar eminances, tending to spare the nasolabial folds.
2. Discoid rash: erythematous, raised patches with adherent keratotic scaling and follicular plugging.
3. Photosensitivity:skin rash as a result of unsual reaction to sunlight, as determined by patient history or physician observation.
4. Oral ulcers: oral or nasopharyngeal ulceration, usually painless, observed by physician.
5. Arthritis:nonerosive arthritis involving two or more peripheral joints, characterized by swelling, tenderness, or effusion.
6. Serositis: pleuritis, by convincing history of pleuritic pain, rub heard by physician, or evidence of pleural effusion, or pericarditis documented by electrocardiography, rub heard by physician, or evidence of pericardial effusion.
7. Renal disorder:persistent proteinuria > 500 mg per 24 hours (0.5 g per day) or > 3+ if quantitation is not performed or cellular casts (may be red blood cell, hemoglobin, granular, tubular, or mixed cellular casts).
8. Neurologic disorder:seizures or psychosis occuring in the absence of offending drugs or known metabolic derangement (e.g. uremia, ketoacidosis, electrolyte imbalance).
9. Hematologic disorder: hemolytic anemia with reticulocytosis;or leukopenia, < 4,000 per mm^3 (4.0×10^9 per L) on two or more occasion, or lymphopenia, < 1,500 per mm^3 (1.5×10^9 per L) on two or more occasions, or thrombocytopenia, <100 $\times 10^3$ per mm^3 (100×10^9 per L) in absence of offending drugs.
10. Immunologic disorder:antibody to double stranded DNA antigen (anti-ds DNA) in abnormal titer; or presence of antibody to Sm nuclear antigen (anti-Sm); or positive finding of antiphospholipid antibody based on an abnormal serum level of IgG or IgM anticardiolipin antibodies, a positive test result for lupus anticoagulant using a standard method, or a false-positive serologic test for syphillis that is known to be positive for at least 6 months and is confirmed by negative *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test.
11. Antinuclear antibodies an abnormal antinuclear antibody titer by immunofluorescence or equivalent assay at any time and in the absence of drugs known to be associated with drug-induced lupus.

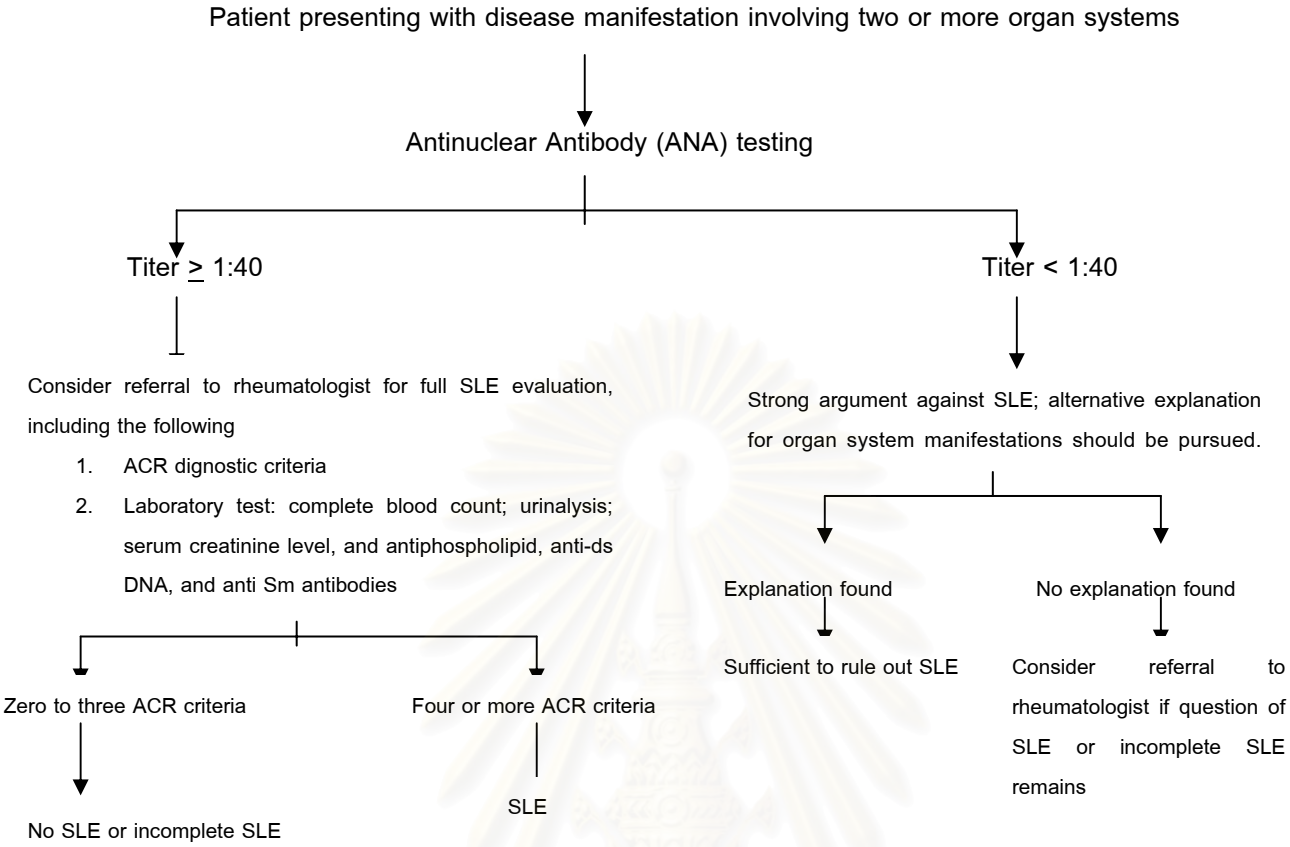


Figure 1. Algorithm for diagnosis of SLE [10]

Table 4 Novel treatments for SLE [21]

Anticytokine therapies

- Anti TNFα
- Anti-interleukin 1-receptor:anakinra
- Anti-interleukin 10
- Anti-interleukin 6 receptor
- Anti-interferon alpha
- Antilymphocyte stimulator (Blys)

Costimulation inhibition

- AntiCD154
- CTLA4Ig:abatacept

B-cell anergy

- LJP394: abetimus

B-cell depletion

- Anti-CD20: rituximab
- Anti-CD22:eprtuzumab

Other techniques

- Immunoadsorption
- AntiC5a
- T cell vaccination
- δ chain transfection
- Peptide therapies: edratide targeting antiDNA idiotypes

2. N-acetylcysteine (NAC)

N-acetylcysteine is a derivative of the amino acid cysteine.

2. 1 Chemical Properties and Pharmacokinetics

Chemical Formula: $C_5H_9NO_3S$ and the chemical structure is illustrated in Figure 2. [1]

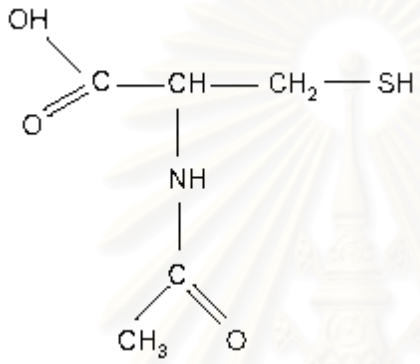


Figure 2. The Chemical Structure of N-acetylcysteine

Molecular Weight: 163.2 g/mol

The intravenous route offers a more rapid onset of action in chronic bronchitis and bronchiectasis compared with oral and intramuscular routes.

Absorption: Oral bioavailability is 6-10%.[1,4] The similar bioavailability is observed for a single 600 mg dose and 200 mg dose.[4] Conflicting data exist concerning the effect of activated charcoal on oral bioavailability. One study found no difference in total area under the curve [27], while *in vitro* data demonstrated significant adsorption of the drug to activated charcoal.[28]

Distribution: Distribution half- life = 0.11 L/kg/hour

Volume of distribution (Vd) = 0.33 to 0.47 L/kg

Metabolism: Only L-NAC is active; L-NAC is metabolized to cysteine and glutathione. The metabolism of NAC is presented in Figure 3.[4]

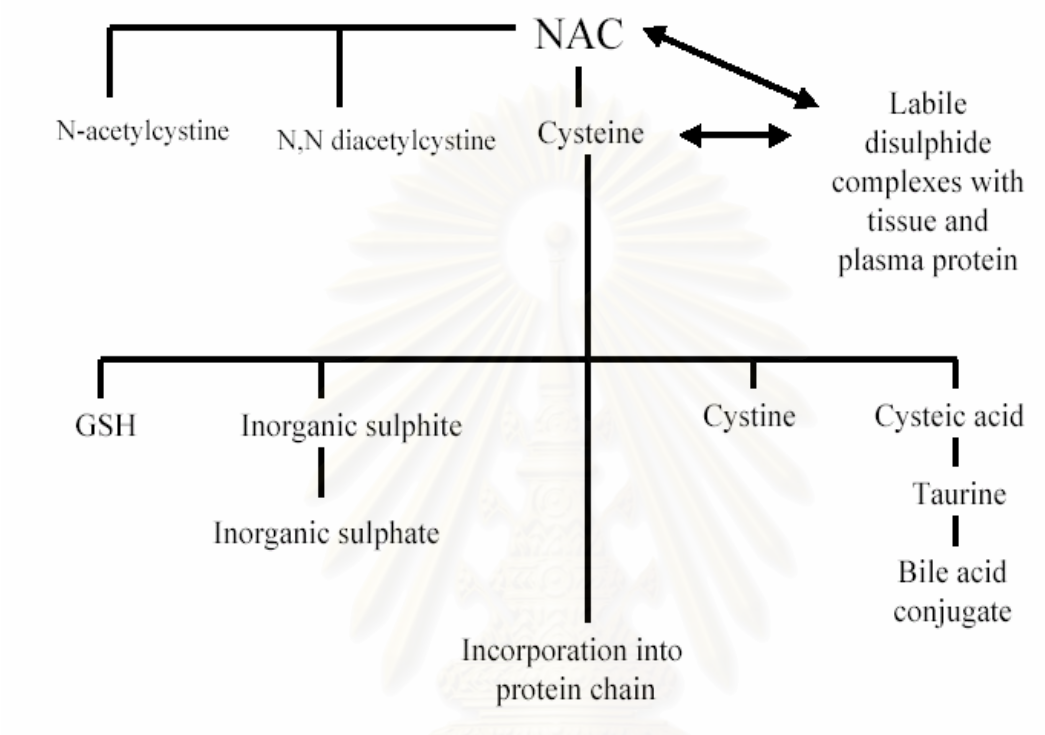


Figure 3. Metabolism of N-acetylcysteine [4]

Excretion: NAC is excreted via renal about 30%. Renal clearance is 0.21L/hour/kg. Total body clearance is 6.5 L/hr. However, it is decreased to 4.5 L/hr in patients with hepatic cirrhosis [29]. Elimination half life ranges from 3 to 6 hr. [4, 30, 31]

2.2 Pharmacokinetics and pharmacodynamic study of NAC

The pharmacokinetics study of NAC has been established since twentieth century (1900's).[30-33] Study on the disposition and kinetics of intravenous NAC in patients with paracetamol overdose showed that the mean maximum plasma concentration after the loading dose was 554 mg/L.[4] Rapidly decline of NAC was occurred and after 12 hour, a mean steady state concentration was 35 mg/L.[4] The summary of pharmacokinetics of NAC was presented in Table 5.[4, 31]

Table 5 Summary of Pharmacokinetics of NAC [4, 31]

Pharmacokinetic parameters (PK)	Value
C_{max} (mg/dL)	0.35-3.47
T_{max} (h)	0.5-3
$T_{1/2}$ (h)	2-6
Vd (L/kg)	0.33-0.47
CL (L/h*kg)	0.84 (CL_t) 0.19-0.211 (CL_R)
F	10%

C_{max}= maximum concentration, T_{max}= time to maximum concentration, $T_{1/2}$= half-life

Vd = volume of distribution, CL = clearance, F = bioavailability

The alteration of NAC pharmacokinetics was occurred in patients with chronic liver disease (cirrhosis).[29] The result showed that the area under concentration time curve (AUC) was significantly increased in cirrhosis patients when compared with those observed in normal control (152.34 $\pm$ 50.38 mg/L*h vs 93.86 $\pm$ 9.6 mg/L*h).[29] The clearance of NAC was reduced when compare with controls (4.52 $\pm$ 1.87 L/h vs 6.47 $\pm$ 0.78 L/h).[29]

The effect of NAC pharmacodynamic was observed. The various effect of NAC on oxidative reactions was proposed by the study in cell culture and animal model. The brief molecular mechanism was presented in Figure 4.[5]

Focus on Pharmacokinetics/Pharmacodynamics (PK/PD) relationship study, the pharmacokinetics and pharmacodynamic of NAC was performed as phase 1 trial in patients who had malignant tumor. Twenty six subjects were recruited in this study. Seven points of blood sampling (0.5, 1, 2, 3, 4, 6, and 8 h after administration) were done to measure NAC level for pharmacokinetics studies, dose escalation was performed at 400 mg, 800 mg ,1.6 g and 3.2 g of NAC. Glutathione level in peripheral blood lymphocyte (PBL) at 1 and 4 h were measured for pharmacodynamic evaluation of 800 mg/day of NAC. The evaluation of pharmacodynamic was performed right after first dose, at the end of first month, secound months and the end of sixth months. The results showed that the administration of NAC did not elevated glutathione level in PBL.[34] However, the 20% increase of GSH in PBL was occurred in 30% or more subjects.[34] The correlation between C_{max}, AUC and the percentage of GSH in PBL was not shown. The researchers concluded that oral administration of 800 mg/m^2 of NAC could modulate the pool of GSH in some subjects but the increment was transient phenomenon.[34]

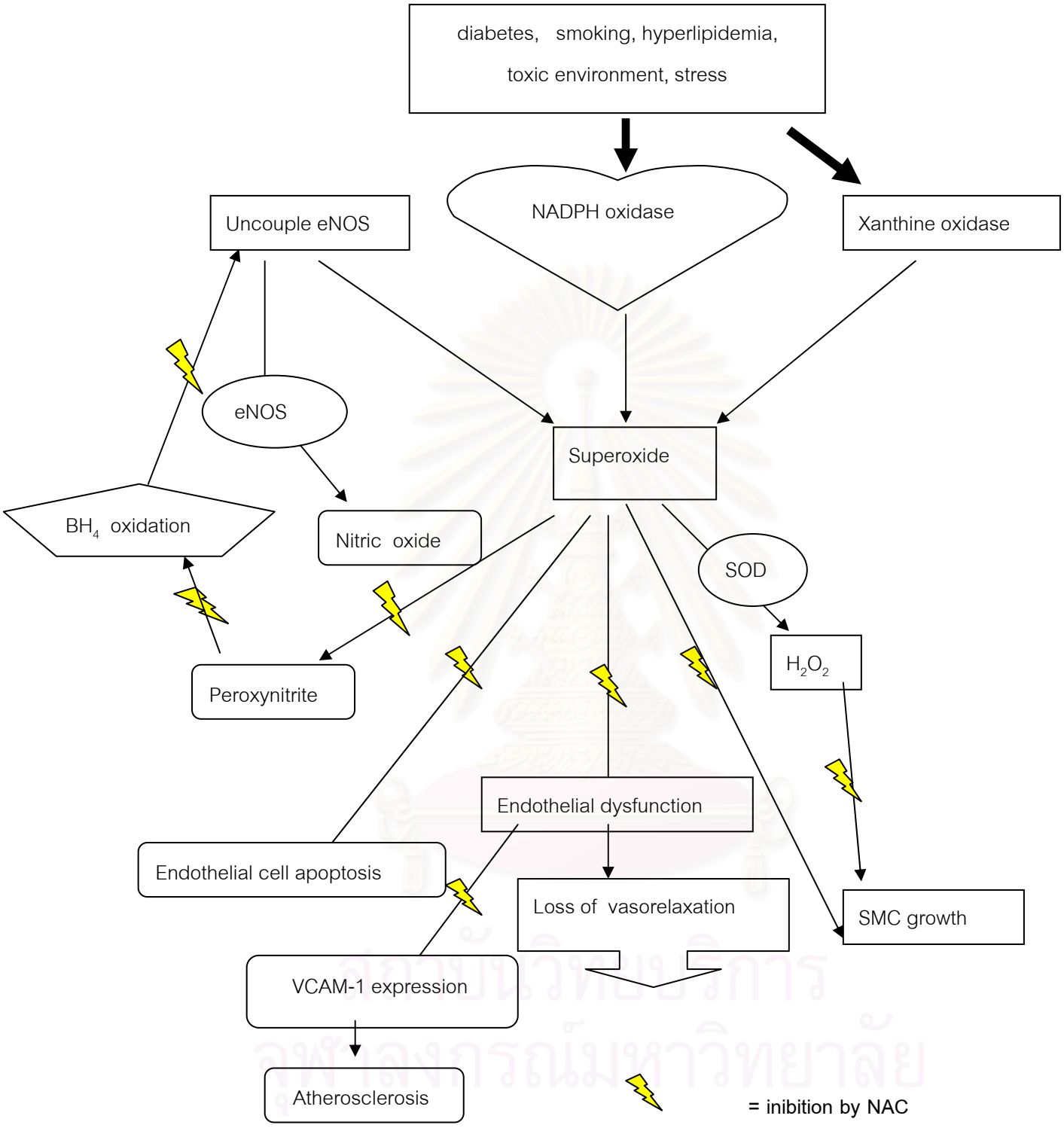


Figure 4 The scheme of molecular action of NAC (BH_4 = tetrahydrobioterin, SOD = superoxide dismutase, e-NOS= endothelial nitric oxide synthase, SMC = smooth muscle cells, VCAM-1= vascular cell adhesion molecule-1) [5]

2.3. Clinical Application of NAC

2.3.1. Respiratory disease: Several studies in animal and human have indicated that NAC can be used for expectoration, reduction of cough severity and diaphragm fatigue.[1, 3, 35, 36] Some small studies revealed that administration of NAC 600 mg three time daily in patients with alveolitis could improve both lung function and glutathione level. [36] However, the effective of NAC in chronic bronchitis, severe airway obstruction, and cystic fibrosis still have been questionable and further studies are needed.

2.3.2 HIV infection: Several studies confirmed that HIV infected patients exhibited low GSH and cysteine level and NAC is a challenging drug to improve GSH status in these patients.[3, 35, 37-40] Researches reveal that NAC can enhancing T cell colony formation and blocking NF kappa B expression [5, 40, 41]

2.3.3. Cancer: The administration of NAC may effect in the prevention of cancer and sometimes could be combined in the treatment of some forms of cancer. However, the recent information have been only preliminary researches in cell culture and animal studies. The experimentally induced DNA damage can be completely blocked by NAC and evidence also indicated that NAC can protect bone marrow cell from the growth inhibitory effect of chloramphenicol and thiamphenicol.[3] NAC has been presented antimutagenic activity.[3] Administration of NAC can also reduce the incidence of experimentally induced intestinal tumor.[3] The investigation of the effect of NAC on GSH metabolism and on biotransformation of carcinogenic and/or mutagenic compounds was performed by De Flora et al.[42] The *in vitro* results showed counteract effect on mutagenicity and at high concentration, completely inhibited the mutagenicity of procarcinogen.[43] *In vivo* , NAC can also inhibit the mutagenicity of the number of compound and can inhibit the induction of tumors by some carcinogens.[42] Combination of doxorubicin and NAC under various experimental conditions can be highly effective, apparently working

synergistically to reduce tumor formation and prevent metastasis.[44] Surprisingly, NAC pretreatment and diminished the cardiac toxicity in mice.[44] NAC also increase the non-protein SH content of P388 leukemia cell about three fold without negative effect.[45] Evidence indicates that NAC did not interfere the effect of killing of tumor cell by x-ray or bleomycin[46], although NAC could help protecting against toxicity resulting from X-ray or chemotherapeutic agents.[1, 3, 34, 47]

Kobrisky et al.[48] found that 19 patients with advanced cancer who had moderate fatigue, anorexia and weight loss were rescued by administration of NAC and high dose acetaminophen. The 15.8 percent of partial response rate was observed.[48]

2.3.4 Influenza: The effect of NAC in influenza have been studied. Total 262 subjects of both sexes were received either placebo or NAC 600 mg twice daily for six months.[49] Although frequency seroconversion was similar in the 2 groups, NAC treatment decreased both the frequency and severity of influenza like syndrome and the length of time confind to bed. De Flora et al. concluded that NAC did not prevent Influenza infection but only reduced incidence of clinically apparent of diseases.[49]

2.3.5 Heart disease: NAC may represent several therapeutic effect associated with cardiac disease. NAC seems to have positive effect on homocysteine and lipoprotein (a) level for protection against ischemic and reperfusion damage, and enhance aspects of the effectiveness of nitroglycerine (NTG).[50] The study on administration of 2 grams daily for four weeks followed by 4 grams daily for four weeks for 2 patients with high lipoprotein (a) level, reported 70 percent reduction in these individuals (reduction of plasma lipoprotein-A) from 58 to 20 mg/dL and from 59 to 18 mg/dL.[50] In contrast , Wiklund et al.[51] did not find the NAC effect on lipoprotein-A but its effect on plasma homocysteine. NAC can reduce homocysteine level by 45%.[51] The study by Bostom et al.[52] also supported the effect of NAC on homocysteine by

showing that NAC could reduce 16 % total plasma homocysteine in non fasting pre-hemodialysis patients.[52]

There were few studies on the effect of NAC in ischemic and perfusion injury in acute myocardial infarction.[53] Infusion of NAC for 1 hour before ischemia increase tissue GSH by 38%.[53] The ischemic induced by the decrease in GSH and protein SH levels was prevented by the administration of NAC.[53]

NAC, in the combination with NTG and streptokinase, was associated with significantly less oxidative stress, more rapid reperfusion and better preservation of the left ventricular function in patients with myocardial infarction. After that, there have been more confirmation study in effect of NAC on cardiovascular disease, especially in myocardial infarction.[3, 54]

2.3.5 Cigarette smoking: Oral administration of NAC may benefit in patient who had heavy smoking or exposed to second–hand smoke (called secondary smoker). Supplementation of NAC can inhibit smoking induced mucus cell hyperplasia and epithelial hypertrophy. [55, 56]

The data suggest that oral administration of NAC can decrease inflammation in the bronchoalveolar cell of smoker.[55] Administration 200 mg of NAC three times a day can prevent the decline of aveolar lymphocytes proportion, the decrease in phagocytic activity, and leukotriene B4 production ability by aveolar macrophage in smokers.[57]

2.3.6 Other effects: The known effect of NAC in the treatment of acetaminophen overdose is published elsewhere.[58-62] Administration of NAC (intravenous or orally) within 24 hours of paracetamol consumption is effective at preventing hepatotoxicity.

Moreover, antioxidant activity of NAC was useful in the treatment of heavy metal intoxication by chelating. [63-65]

2.3.7 NAC and autoimmune diseases or renal disease

2.3.7.1 NAC and Multiple sclerosis (MS)

There is a marked elevation of the cytokine tumor necrosis factor α (TNFα) in active multiple sclerosis (MS), and a correlation exists between cerebrospinal fluid (CSF) levels of TNFα and the severity and progression of disease. With cytokine activation, the increase in free radical production has been demonstrated in MS. NAC is a free radical scavenger and inhibits toxicity of TNFα[66] and in the experimental autoimmune encephalomyelitis (EAE) animal model[66] of MS, the development of MS-like pathology was inhibited. Ten patients with MS took NAC for a period of up to 16 months. Because of the relapsing-remitting course of the disease occurring in many MS patients, it was difficult to ascertain efficacy of NAC in these preliminary studies.[66] However, two MS patients with longstanding inability to speak coherently had a rather dramatic irnprovement in speech shortly after starting the drug. Controlled trials are necessary to ascertain if NAC can decrease the number of exacerbations in MS. [66]

2.3.7.2 NAC and Idiopathic Pulmanary Fibrocis (IPF)

There was clinical study about high dose NAC in idiopathic pulmonary fibrosis.[36] Demedts et al. concluded that treatment with 1800 mg of NAC in the combination with prednisolone and azathioprine preseved vital capacity and diffusion capacity (DL_{50}) in patient with idiopathic pulmonary fibrosis.[36]

2.3.7.3 NAC and Sjogren's syndrome

NAC may have benefit on ocular symptoms. Walter et al.[67]studied the therapeutic effect of NAC (200mg three time per day) in 26 patients with primary or secondary Sjogren's syndrome for four week study. Six of twenty patients reported improvement of occular soreness (p=0.004) and oculars irritability (p-0.006) following supplementation of NAC. Halitosis (p-0.033) and day time thirst (p=0.033) were also improved following the NAC supplementation.

2.3.7.4 NAC and renal disease (contrast media induce nephrotoxicity)

NAC has action relevant to radio contrast induced nephropathy (RCIN) that include vasodilatation, enhancement of renal medularly blood flow and antioxidant properties. The drug's pharmacokinetics is remarkable for almost complete first pass metabolism after oral administration, resulting no free drug in circulation.[68] After IV administration, the reaction with tissue and plasma protein greatly limit the amount of free drug in circulation. The primary mechanism may through L-cysteine, a cellular source of GSH production. Controversy result about the benefit of NAC in preventive of RCIN has still were observed as shown in Table 6. [68, 69]

2.3.8 NAC and SLE

This is a first study about effect of NAC in SLE patients. However, in animal study [9] showed that the administration of NAC 250 mg/kg/day in female NZB x NZW F1 mouse model of SLE can prolong the survival time as compared to the control (33$\pm$ 2 VS 38$\pm$ 2 wk), even though it was less than cystenine (48$\pm$ 2 wk)[9].

Table 6 Randomized trial evaluating NAC for the prevention of radiocontrast-induced nephropathy [68]

Lead Author	n	Placebo Group RCIN (%)	Renal Entry criteria Scr (mg/dL)/CrCl (ml/min)	Oral NAC dose	Contrast Procedure	Country
Positive studies						
Baker[70]	80	21		IV dose	Coronary cath +/- PIC	United Kingdom
Diaz-Sandoval[71]	54	45	>1.4/<50	TP	Coronary cath +/- PIC	United States
Kay[72]	200	12.2	>1.2/<60	TP	Coronary cath +/- PIC	China
Shyu[73]	121	24.6	>2/<40	400 mg bid X 2d	Coronary cath +/- PIC	Taiwan
Tepel[74]	83	21.4	>1.2/<50	TP	CT	Germany
Negative studies						
Allaqaband[75]	85	15	>1.6/<60	TP	Coronary cath +/- PIC	United States
Boccalandro[76]	179	12.3	>1.2/<50	TP	Coronary cath +/- PIC	United States
Briguori[77]	183	11	>1.2/<70	TP	Coronary cath +/- PIC And peripheral angio	Italy
Durham[78]	79	22	>1..7	1200 mg bid X1d	Coronary cath +/- PIC	United States
Goldenberg[79]	80	7.7	>1.5	600 mg tid x 2d	Coronary cath +/- PIC	Isarael
Loutrianakis[80]	47	13	>1.5	TP	Coronary cath +/- PIC	United States
Oldemeyer[81]	96	6..4	Crcl < 50m:/min	1500 mg bid x 2d	Coronary cath +/- PIC	United States
Vallero[82]	20	0	>1.2	Vallerno TP		Italy

TP = Tepel protocal, 600 mg orally twice daily on the day before and the day of the procedure

PIC = Percutaneous coronary intervention

3. Glutathione Malondialdehyde and oxidative system

3.1 Glutathione

Glutathione is a ubiquitous cellular antioxidant. Three main functions of glutathione have been observed [1, 2, 83]:

1. GSH is an extreamly important cell protector. It directly quenches reactive hydroxyl free radicals, other oxygen-centered free radicals and radical centers on DNA and other biomolecules. GSH is a vital guard of skin, lens, cornea, and retina against radiation damage and the biochemical foundation of P450 detoxification in the liver, kidneys, lungs, intestinal epithelia and other organs.

2. GSH is the essential cofactor for many enzymes which necessary require thiol-reducing equivalents and helps to mainten redox-sensitive site on enzymes in reduced state.

3. GSH and its metabolite also interface with energetics and neurotransmitter synthesis. GSH availability down-regulates the pro-inflammatory potential of leukotrienes and eicosanoids. Low concentration of GSH have been involved in numerous pathological conditions such as inherited deficiency, HIV infection /immunity, liver cirrhosis, Inflammation, pulmonary disease, Crohn's disease, gastrointestinal inflammation, circulation, neurodegerative/CNS disorders, and aging. [1, 2, 83-85]

The GSH level in human tissue normally range from 0.1-10 mM, highest concentration in liver (up to 10 mM) and in the spleen, kidney, lens, erythrocyte and leukocytes. Plasma concentration is in the micromolar range. [1, 2, 84, 85]

The attempt to measure GSH level in human tissues and biological fluids has been developed; many methods are implemented such as spectrophotometer assay, fluorometric assays, biochemical assays, capillary electrophoresis and HPLC. [84] The normal values for total, free, and reduced glutathione in plasma and in whole blood varied from one laboratory to another. The variability may be related to different methodology, differences in sample processing and the way to select the subjects who are under the influence of various factors affecting the plasma/blood glutathione concentrations. [84, 86, 87]

3.2 Malondialdehyde (MDA)

Malondialdehyde (MDA) is the principal and most frequent studied product of polyunsaturated fatty acid peroxidation. The several methods have been developed to quantified this molecule in order to evaluate the level of oxidative stress in human. The main source of MDA in biological samples is the peroxidation of polyunsaturated fatty acid. However, MDA can also be formed *in vivo* by enzymeatic processes from various protaglandins. [88, 89]

In the past, MDA has been recognized as a relevant lipid peroxidation marker and as such determination of MDA levels in biological samples from subjects affected by several diseases has been widely utilized. The increasing in MDA level has been discovered in various diseases such as cancer [90, 91], preclampsia [92], diabetes [93], cardiovascular diseases [94], dementia [95], and autoimmune diseases. [96, 97] Most assays to determine MDA have been developed on the basis of its derivertization with thiobarbituric acid (TBA). The condensation of these two molecules rise its absorbility adduct which can be simply assessed with spectrophotometer. [89]

Plasma MDA or TBARS concentrations obtained with the methods developed from 1970 to 1995 varied in vary wide range (from 0-50 μM). [88, 89] TBARS measurement continue to be assessed in clinical trial and often give positive result, apperently demonstrating level of oxidative stress higher in pathological than in healthy. Fluorometry , HPLC, GCMS with acidic condition of sample have been used. [88]

3.3 Oxidative system [98]

Increase oxidative /nitrosative stresss formally describes a condition in which cellular antioxidation defense are inadequate to completely in-activate the reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to excessive production of ROS/RNS, loss of antioxidant defense or both. [98]

A major consequence of oxidative/nitrosative stress is damage to nucleic acid bases, lipids, and protein which can severely compromise cell health and viability or induce a variety of cellular response through production of secondary reactive species, ultimately leading to cell death by necrosis or apoptosis. The promising oxidative stress biomolecular of oxidative damage in human disease is presented in Table 7. [98]

Table 7 Oxidative stress biomolecular of oxidative damage in human diseases [98]

Parameters	Diseases
Malodialdehyde (MDA)	Alzhimer disease Asthma Antiphospholipid syndrome Diabetes mellitus
4-Hydroxy 2- Nonenal (HNE)	Alzhimer disease Atherosclerosis Cadiovascular diseases COPD
F2 –Isoprostanes	Acute respiratory distress syndrome (ARDS) Acute chronic alcohol liver disease Alzhimer Chronic kidney disease COPD Cystic fibrosis Diabetes Melitus

Table 7 Oxidative stress biomolecular of oxidative damage in human diseases (continue)

Parameters	Diseases
Decrease in GSH concentration and/or GSH:GSSH ratio	Acute respiratory distress syndrome (ARDS) Alcohol liver disease Alzhimer disease Asthma Cancer Cardiovascular diseases HIV positive Idiopathic pulmonary fibrosis (IPF)
S-Glutathionylated protein	Cataract genesis Diabetes HIV infection Hyperlipidemia
NO_2-Tyr	Acute respiratory distress syndrome (ARDS) Chroronary atery disease Alzhimer Cystic fibrosis

CHAPTER III

METHODOLOGY

Population

The study was performed in SLE patients at Ramathibodi hospital. The participants were asked to write the consent form before enrollment. Study protocol was approved by the hospital ethic committee on April 2005.

Materials

N-acetycysteine preparation

The N-acetylcysteine (Fluimucil®) was kindly provided from SM pharmaceutical company.

Fluimucil A 600 (N-acetylcysteine effervescent tablet 600mg, Lot number 080007/1, Expired: 06/2009)

Chemical materials:

1. 2-Thiobarbituric acid

   (Fluka , Lot & Filling code = 1176252, 23705112, USA)

2. Phosphotungstic acid hydrate

   (Fluka , Lot & Filling code = 1113100, 42604243, USA)

3. MDA Standard (A.C.S Xenon Inc, USA)

4. Glutathione Kit (Telorsu suplied Inc, Bioassay system, USA)

Instruments

1. Microplate reader (Victor 2® Perkin Elmer, Turku, Finland)
2. Electric Analytical Balance (Metler toledo, England)
3. Vortex mixer (Vortex-Genie2® Scientific industries, USA)
4. Refrigerated centrifuge (Beckman High Speed Floor Model J2-MC Digital Centrifuge, Japan)
5. Water bath (Hetofrig CB 60, Julabo Paratherm U8 waterbath, Germany)
6. Disposible syringe 5 mL (Terumo corperation, Japan)
7. Needdle No 22 G X 1" (Terumo corperation, Japan)
8. Polypropylene test tubes 5 mL (Corning Inc, USA)
9. Microcentrifuge tubes 1.5 mL (Corning Inc, USA)
10. Micropipette 200-1,000 μL (Gilson, France)
11. Pipette tips 1,000 μL (Corning Inc, USA)
12. Pipette tips 200 μL (Corning Inc, USA)
13. 96-well polystyrene clear plate , white (Corning Inc, USA)
14. 96-well polystyrene opaque plate , black (Corning Inc, USA)

Methods

This study was divided into 3 parts.

Part 1: **Epidemiology Study**

The retrospective study for the trends of this disease was conducted at department of medicine, Ramathibodi hospital. The prevalence of SLE during 7 years was determined and the pattern of treatment was studied. This study was performed at division of Allergy,Immunology and Rheumatology, Ramathibodi hospital, Bangkok, Thailand. Retrospective data of the patients who visited at the medicine clinic was retrieved from the annual report of the hospital. The number of patients who had definite diagnosis as SLE in medical clinic was obtained from the biomedical information technology service. The data for drug pattern study was collected during January 2000 to December 2006 from medical records of patients who visit the medicine clinic.

The prevalence of SLE was calculated from the following formula

$$\text{prevalence of SLE} = \frac{\text{The number of patients who are diagnosed as SLE at medicine clinic}}{\text{The number of patients who visit medicine clinic}}$$

The number of patient's medical records that would be recruited in prevalence study was calculated by using the following formula [99].

$$N = \frac{Z_{\alpha/2}{}^2 pq}{d^2} = \frac{1.96^2 x 0.007 x 0.993}{0.0007^2} = 383.89$$

N = total number of sample

$Z_{\alpha/2}$ = the probability of Z distribution at $\alpha/2$

P = the prevalence of SLE (previous study [13] , p = 0.007

q = 1-p (= 0.993)

d = the deviation from the true prevalence (0.01 x p) = 0.0007

α = statistical significant level (=0.05)

Substitution this number to above formula resulted of 383.89 or 400 patients' medical records that require to study the pattern of SLE therapeutic medication. In addition, 1-year cross-sectional study of drug pattern of SLE patients from these medical records was performed.

The therapeutic outcomes were classified as remission or inactive, improve, and active by using Systemic Lupus Erythematosus Disease Acitivity Index (SLEDAI) which were defined as followed [100]

Remission or in active: SLEDAI =0

Improve: reduction of SLEDAI >3

Active: change in SLEDAI 1-3

The therapeutic outcomes depended on physician's decision.

Part II: **Distribution and comparision of oxidative status in SLE patients with different severity.**

In order to confirm the oxidative theory, oxidative status in SLE patients with different severity (during active and non active status) was investigated. The severity was classified according to the following definition[101]:

Mild SLE : Characterized by arthritis, arthralgia, myalgia, fatigue, mild mucocutaneous involvement, low-grade fever, mild serositis, lupus headache. (SLEDAI <10)

Moderate SLE: Characterised by high-grade fever, toxaemia, severe mucocutaneous manifestations, marked photosensitivity, moderate to severe myocarditis, mesangioproliferative or minimal change lupus nephritis, haemolytic anaemia and thrombocytopenia. (SLEDAI 10-20)

Severe SLE: Characterised by organ/life-threatening features such as focal/diffuse proliferative glomerulonephritis with or without azotaemia/hypertension, lupus cerebritis with recurrent seizures, acute confusional state, coma; systemic necrotizing vasculitis such as one causing peripheral gangrene, GI bleeding or mononeuritis multiplex. (SLEDAI>20)

The voluntary healthy subjects were also investigated as controls. Comparision of oxidative stress among SLE patients with different severity was performed. Oxidative stress between active and non active status, was also be compared, the sample size for each arm was calculated from the following formula [99]

$$n = \frac{(Z_{\alpha} + Z_{\beta})^2 \sigma^2 x2}{d^2}$$

α = 0.05, β = 0.2 , d = the effect size =0.026. [102]

$$n = \frac{(1.96 + 0.84)^2 0.028^2 x 2}{0.026^2} = 18.18$$

Therefore, minimum sample size in each arm is 20.

In addition, the severity was also classified by using SLE disease activity index (SLEDAI) as the following [103]

Mild: SLEDAI score <10

Moderate: SLEDAI score 10 – 20

Severe: SLEDAI score >20

The association between oxidative status and severity of SLE was determined. In addition, relationship between oxidative status and SLEDAI score were also calculated.

Determination of oxidative status

According to the oxidative stress theory, the determination of malondialdehyde (MDA) which represented lipid peroxidation and glutathione (GSH) which represented anti-oxidative status should be performed.

Sample preparation

Blood sample was collected in EDTA tube and centrifuged at 3,000 rpm 2°C within 10 minutes for plasma separation. The plasma was kept at –20 $^{\circ}$C and was analyzed for MDA and GSH within 2 weeks.

Determination of MDA

The measurement of plasma MDA was conducted as followed; 2 mL of 1/12 N H_2SO_4 and 0.3 mL of 10% phosphotungstic acid was added in 100 μL of plasma. The mixture was incubated at room temperature for 10 minutes and centrifuged at 2,500 g for 3 minutes. After that pellet was separated and supernatant was transferred to test tube, 1mL of 0.67% thiobarbituric acid (TBA) and 2 mL of distill water were added to the transferred supernatant. The sample was incubated at 100°C for 1 hour and the concentration was measured by fluorometry with the excitation and emission wavelength were 515 nm and 553 mn, respectively.

Method validation

Linearity: Seven concentrations of standard MDA were prepared; 0, 0.1, 0.3, 0.5, 1.0 , 2.0, and 3.0 μM, respectively. Linearity plot between MDA concentration and absorbance intensity was performed, and standard curve equation was calculated with coefficient of determination (r^2).

Precision and Accuracy: Three standard concentrations of MDA were selected for precision and accuracy testing at 0.1, 0.5, and 3.0 μM, respectively. Five replicated of each concentrations were used for intra-day precision while 15 replicated within 3 consecutive days were used for inter-day precision and percent of coefficient of variation (%CV) was reported. The acceptance criteria for %CV was less than 15% except the lowest concentration was allowed to 20%. The accuracy calculation was followed by the following equation

$$\%\,\text{inaccuracy} = \frac{\left[\text{Concentration added - Concentration found}\right] \text{x } 100}{\text{Concentration added}}$$

Recovery: The recovery of this method for each 3 concentrations (0.1, 0.5, and 3.0 μM) was calculated by the following equation

$$\%\,\text{recovery} = \frac{\left[\text{Concentration found after analytical process}\right] \text{x } 100}{\text{Concentration added}}$$

Determination of Glutathione (GSH)

The Glutathione test kit was used for determination of Glutathione in plasma. The method of determination was followed using the 55'-dithiobis-(2-nitrobenzoic acid) or DTNB principle. The plasma was diluted 10 times before analyzed. One hundred and fifty micro liters of diluted plasma was mixed with the same volume of reagent A (phosphoric acid) in microcentrifuge tube. The mixture was centrifuged at 13,400 rpm with 2 minutes. The 200 μL of supernatant was transferred

into 96-welled plate, then 100 μL of reagent B (DTNB) was added. The mixture was allowed to incubate for 20-25 minutes, final detection was performed at 450 nm.

Method validation

Linearity: Eight concentrations of standard MDA were prepared; 0, 10, 20, 30, 40 , 60, 80 and 100 μM, respectively. Linearity plot between GSH concentration and absorbance intensity was performed, and standard curve equation was calculated with coefficient of determination (r^2).

Precision and Accuracy: Three standard concentrations of GSH were selected for precision and accuracy testing; 5.0, 50, and 90 μM, respectively. Three replicated of each concentrations were used for intra-day precision while 9 replicated within 3 consecutive days were used for inter-day precision and percent of coefficient of variation (%CV) was reported. The acceptance criteria for %CV was less than 15% except the lowest concentration was allowed to 20%. The accuracy calculation was calculated using the following equation

$$\%\,\text{inaccuracy} = \frac{\left|\,\text{Concentration added} - \text{Concentration found}\,\right| \times 100}{\text{Concentration added}}$$

Recovery: The recovery for each 3 concentrations (5.0, 50, and 90 μM) was calculated using the following equation

$$\%\,\text{recovery} = \frac{\left|\,\text{Concentration found after analytical process}\,\right| \times 100}{\text{Concentration added}}$$

Part 3: Study the effect of NAC administration as adjunctive therapy in mild SLE

Subjects

The patients were recruited according to the following criteria:

Inclusion criteria

1. Males or females whose ages more than 18 year-old who were diagnosed as SLE according to American College of Rheumatology (ACR) criteria and severity is mild according to the definition already mention in Phase II study.

2. Patients received standard treatment for mild SLE. All administered drugs will be recorded.

3. Patients were willing to be included in the study and signed the inform consent.

Exclusion criteria

The patients were exclude if they:

1. were poor compliance with drug regimens
2. were pregnancy and lactation
3. had hypersensitivity or were intolerance to NAC
4. had severe infections or liver diseases
5. were considered by physician as inappropriate to be included in this study.

The process was carried out by the following steps

1. The participants were randomly allocated into 2 groups; control and treatment groups. The following Pharmacodynamic parameters (PD) at baseline for every patients were recorded which were

- Glutathione levels
- Malondialdehyde levels
- SLEDAI (Systemic Lupus Erythematosus Disease Activity Index)

Sample size calculation

Because there is no previous study to evaluate the effect of N-acetylcysteine in SLE patients. Therefore, σ^2 from previous clinical study on lipid peroxidation or oxidative stress in SLE was used[26]. Then from the following formula,[99] sample size was calculated based on clinical outcome measurement.

$$n = \frac{(Z_\alpha + Z_\beta)^2 \sigma^2 x 2}{d^2}$$

α = 0.05, β = 0.2 , d = the effect size of primary outcome =0.026 [102].

$$n = \frac{(1.96 + 0.84)^2 0.028^2 x 2}{0.026^2} = 18.18$$

Therefore, the minimum sample size in each arm is about 20.

2. since SLE patients also received immunosuppressive drugs.

since SLE patients also received immunosuppressive drugs, they were assigned to receive 600 mg tid of N-acetylcysteine; the effective dose that reported in alveolitis patients who received maintenance immunosuppression [3], since SLE patients also received immunosuppressive drugs.

3. Pharmacodynamic monitoring

Pharmacodynamics (PD)

The follow up was designed every 2 weeks for acute phase and every month for maintenance phase. The effect of NAC adjunctive therapy was evaluated during 6 months. The incidence of disease relapse of the disease was also be observed during maintenance phase. Five milliliters of blood samples were collected at each visit. Glutathione (GSH) and malondialdehyde (MDA) levels were analyzed.

The adverse events were closely observed and recorded. Narajo's algorithm was used for evaluation of adverse drug reactions. Whenever any adverse event occured, the management was implemented immediately. In case of the serious adverse effect, the patients was considered to withdraw from the study.

Statistical analysis

In phase I study, descriptive statistic was used such as percentage, mean, standard deviation.

In Phase II study, the association between oxidative stress status (glutathione and malondialdehyde level) and severity of disease, include SLEDAI was determined by Pearson's correlation. Oxidative stress status among different severity of SLE was compared using the analysis of variance (ANOVA) and multiple comparison. The regression analysis was used and regression equation was proposed.

In Phase III study, glutathione and malondialdehyde level at various time after treatment of the 2 groups were compared using ANOVA and t-test in order to determine efficacy of NAC.

All statistical significant level (α) was set at 0.05.

CHAPTER IV

RESULTS

Epidemiology Study

Prevalence of SLE during 7 years (2000-2006)

During 7 years (2000-2006), there was an increase in prevalence of SLE at Ramathibodi hospital from 231 to 553 as shown in Table 8 and Figure 5, respectively.

The characteristics of SLE patients is present in Table 9. Most patients were female with average of age of to 39.65 $\pm$ 10.55 years and had average duration of disease at 12.25 $\pm$ 6.22 years. Most of them had moderate severity.

Pattern of drug therapy regimens

As presented in Figure 6 and Table 10, prednisolone was the most common drug used din SLE patients. Antimalarials and immunosupressive agents were also frequently used. Combination drug therapy strategies was the most frequently selected (41.64%, N=137).

According to combination drug therapies, the most frequently combinations used was prednisolone combined with hydroxychloroquine (16.79%), prednisolone combined with azathioprine (16.06%) and prednisolone combined with chloroquine (13.87%). For three drugs combination, prednisolone + azathioprine + hydroxychloroquine (14.60%) was frequently used. Most of the combination regimens was prednisolone based (97.09%). Only about 3 percent was non-prednisolone content combination.

Consider of therapeutic outcomes, the results are presented in Table 11 (a and b). Almost half of patients have followed up at division of allergy immunology and rheumatology. More than 75% have remission or inactive (documented by physicians). If patients loss follow up, they were excluded. The percentage of remission or inactive was 88.65%. The percentage of patients who had been still active was less than 5.

Table 8. Prevalence of SLE patients in each year (2000-2006)

Year	No of SLE patients	No. of hospital visitors	Frequency (per 100,000 visitors)
2000	514	192,376	267
2001	518	200,139	259
2002	479	207,221	231
2003	750	269,973	278
2004	762	237,322	321
2005	1375	251,490	547
2006	1448	262,015	553

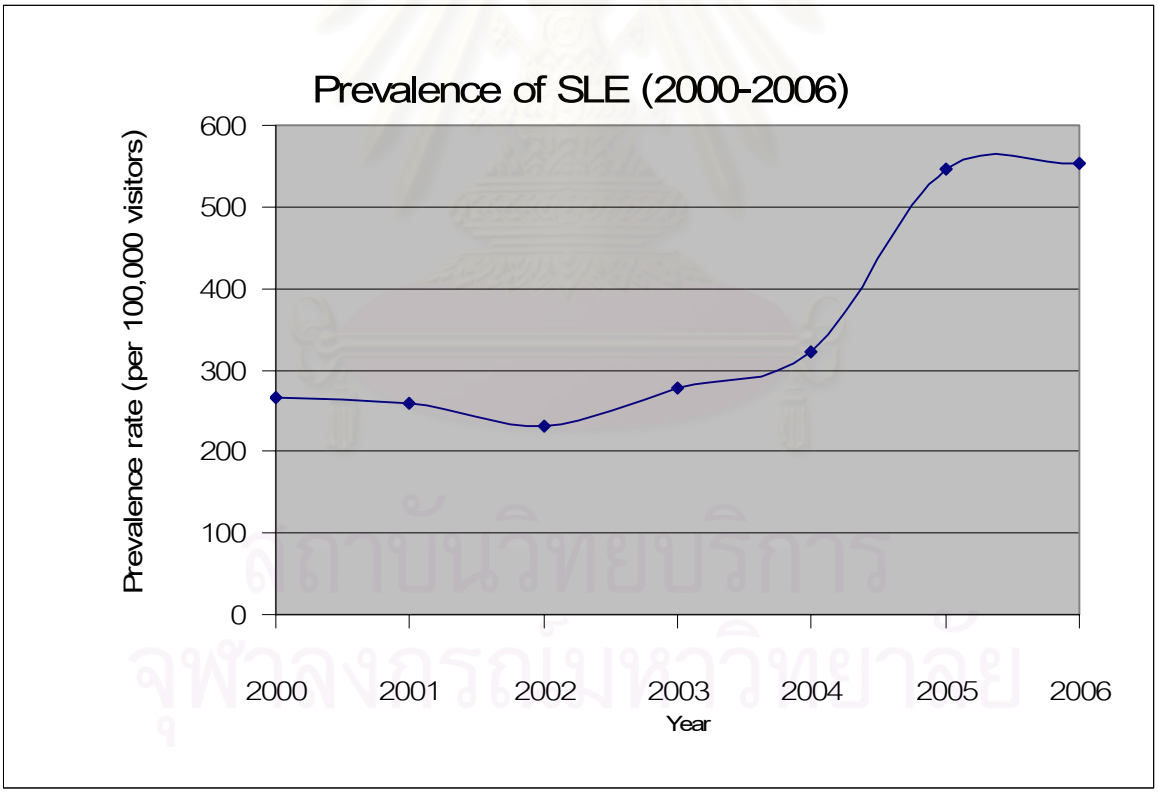


Figure 5 The prevalence rate of SLE (cases per 100,000) during 2000-2006.

Table 9 Characteristic of SLE patients

Characteristic	N =329	%
Gender		
Male	3	0.9
Female	326	99.1
Age (mean ± SD), year	39.65 ± 10.55	
SLE duration (mean ± SD), year	12.25 ± 6.22	
Severity		
Mild	123	37.4
Moderate	174	52.9
Severe	32	9.7

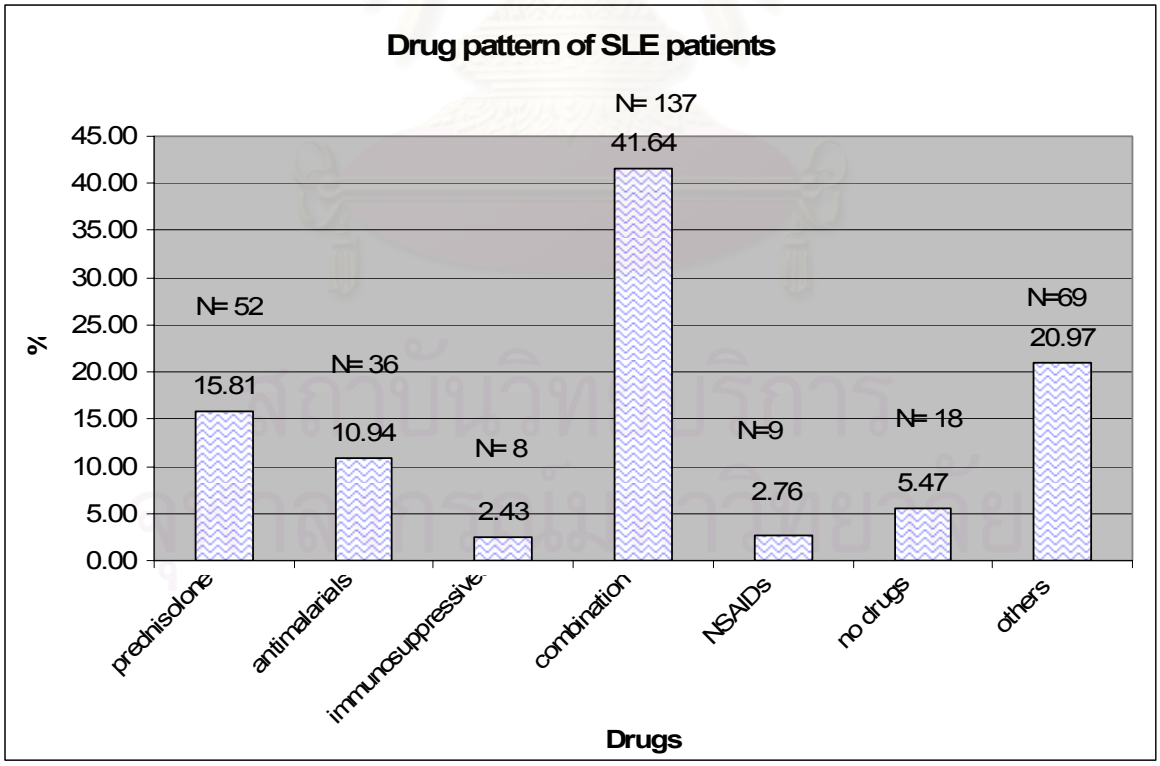


Figure 6 Drug therapy in SLE patient at Ramathibodi hospital (N=329)

Table 10 Frequency of different combination regimens used in SLE patients

Drugs	n (N=137)	%
Pred + HCq	23	16.79
Pred + AZa	22	16.06
Pred + Cq	19	13.87
Pred + CYP	10	7.30
Pred +NSAIDs	9	6.57
Pred + MTX	1	0.73
Pred + AZa +HCq	20	14.60
Pred + Aza+ Cq	8	5.84
Pred + CYP+ HCq	5	3.65
Pred + NSAIDs+ HCq	5	3.65
Pred + NSAIDs+ Cq	3	2.19
Pred + CYP+ Cq	1	0.73
Pred + Aza+ CysA	1	0.73
Pred + Aza+ MTx	1	0.73
Pred + Aza + MMF	2	1.46
Pred +CYP+MMF	3	2.19
HCq+NSAIDs	2	1.46
Cq+ Aza	1	0.73
AZA + NSAIDs	1	0.73
Total	**137**	**100**

*Pred = prednisolone, Cq = Chloroquine, CYP = Cyclophosphamide,
HCq = Hydroxychloroquine, Aza = Azathioprine, MMF=Mycophenolate mofetil

Table 11 (a) Therapeutic outcomes

Outcomes	n (N=329)	%
Remission or in active	240	75.99
Improved	26	7.90
Active	15	4.6
Loss follow up	47	14.3
Death (Active)	1	0.30
Total	**329**	**100.00**

(b) Drug regimens and outcomes classified as remission or inactive, improved, and active base on drug regimens (N=282)

Drug regimens	Outcomes n (%)			
	Remission or inactive	improve	Active	**Total**
Pred	36 (85.72)	3 (7.14)	3 (7.14)	**42**
HCq	3 (100.00)	0 (0.00)	0 (100.00)	**3**
NSAIDs	9 (100.00)	0 (0.00)	0 (0.00)	**9**
Pred+HCq	18 (85.72)	2 (9.52)	1 (4.76)	**21**
Pred+AZA	20 (95.24)	0 (0.00)	1 (4.76)	**21**
Pred+Cq	14 (82.35)	3 (17.65)	0 (0.00)	**17**
Pred+CYP	4 (66.67)	2 (33.33)	0 (0.00)	**6**
Pred+NSAIDs	4 (50.00)	1 (12.50)	3 (37.50)	**8**
Pred+MTX	1 (100.00)	0 (0.00)	0 (0.00)	**1**
Pred+AZA+HCq	17 (100.00)	0 (0.00)	0 (0.00)	**17**
Pred+AZA+Cq	4 (57.14)	2 (28.57)	1 (14.29)	**7**
Pred+CYP+HCq	4 (100.00)	0 (0.00)	0 (0.00)	**4**
Pred+NSAIDs+HCq	3 (100.00)	0 (0.00)	0 (0.00)	**3**
Pred+NSAIDs+Cq	0 (0.00)	1 (100.00)	0 (0.00)	**1**
Pred+AZA+MMF	2 (100.00)	0 (0.00)	0 (0.00)	**2**
Pred+CYP+MMF	2 (100.00)	0 (0.00)	0 (0.00)	**2**
HCq+NSAIDs	2 (100.00)	0 (0.00)	0 (0.00)	**2**
Other drugs	83 (81.37)	12 (11.76)	7 (6.86)	**102**
No drug	14 (100.00)	0 (0.00)	0 (0.00)	**14**
Total (%)	**240 (85.11)**	**26 (9.22)**	**16 (5.67)**	**282 (100.00)**

Pred = prednisolone, Hcq = Hydroxychloroquine, NSAIDs = nonsteroidal anti-inflammatory drugs, AZA = azathioprine, Cq = chloroquine, CYP = cyclophosphamide

There was no association between serverity and proportion of SLE patients who received and not receive four main drug therapy regimens (prednisolone containing regimens, chloroquine containing regimens, azathioprine containing regimen, and hydroxychloroquine containing regimens) as presented in Table 12. Cyclophosphamide containing regimen showed tendency to be prescribed in early onset of SLE. In addition, the duration of SLE is related to the proportion of SLE patients who receive and not receive azathioprine containing regimens while this association was not observed in prednisolone, cyclophosphamide, chloroquine and hydroxylchloroquine containing regimens as present in Table 13.

Table 12 The association of severity and main drug containing regimens

Drug regimens	Severity (number of cases)			
	Mild N=108 (%)	Moderate N=148 (%)	Severe N=26 (%)	P value
Prednisolone containing regimens				
Received	63 (58.33)	77 (52.02)	14 (51.85)	0.878
Not Received	45 (41.67)	71 (47.97)	12 (48.15)	
Chloroquine containing regimens				
Received	15 (13.89)	15 (10.14)	3 (11.11)	0.766
Not Received	93 (86.11)	133 (89.86)	23 (88.89)	
Cyclophosphamide containing regimens				
Received	2 (1.85)	5 (3.38)	2 (7.41)	0.091
Not Received	106 (98.15)	143 (96.62)	24 (92.59)	
Azahioprine containing regimens				
Received	16 (14.82)	25 (16.89)	2 (7.41)	0.277
Not Received	92 (85.18)	123 (83.11)	24 (92.59)	
Hydroxychloroquine containing regimens				
Received	17 (15.74)	28 (18.92)	4 (14.81)	0.760
Not Received	91 (84.26)	120 (81.08)	22 (85.19)	

Table 13 The association between SLE duration and the main drug regimen used.

SLE duration (years)	Total	Prednisolone containing regimen	Chloroquine containing regimen	Azathioprine containing regimen*	Cychlophosphamide containing regimen	Hydroxychloroquine containing regimen
		Received (%)	Received (%)	Received (%)	Received (%)	Received (%)
3-13	177	96 (54.24)	24(13.54)	36 (20.33)	9 (5.08)	34 (19.43)
14-24	93	45 (48.38)	9 (9.68)	10 (10.75)	3 (3.22)	14 (15.05)
25-36	12	13 (72.22)	0 (0.00)	1 (5.55)	0 (0.00)	1 (5.55)
Total N (%)	282	154 (54.60)	33 (11.70)	47 (16.67)	12 (4.25)	49 (17.37)
p vaule		0.275	0.167	**0.030**	0.483	0.359

* $p<0.05$

Validation of the analytical methods for Glutathione and MDA concentrations

Linearity

Glutathione (GSH)

The linearity of the assay method was demonstrated at concentration between 0 –100 μM, The calibration curve between GSH concentrations and absorbance intensity is illustrated in Figure 7. The linear regression equation was

$$Y = 0.0004 X + 0.0006$$

Where

X = GSH concentration (μM)

Y = Absorbance intensity

With determination coefficient (r^2) = 0.9839

Malondialdehyde (MDA)

The linearity of the MDA assay method was demonstrated between concentration range from 0 –20 μM. The calibration curve between MDA concentrations and absorbance intensity is iillustrated in Figure 8. The linear regression equation was

$$Y = 859.95 X + 25.283$$

Where

X = MDA concentration (μM)

Y = Absorbance intensity

with determination coefficient (r^2) = 0.9969

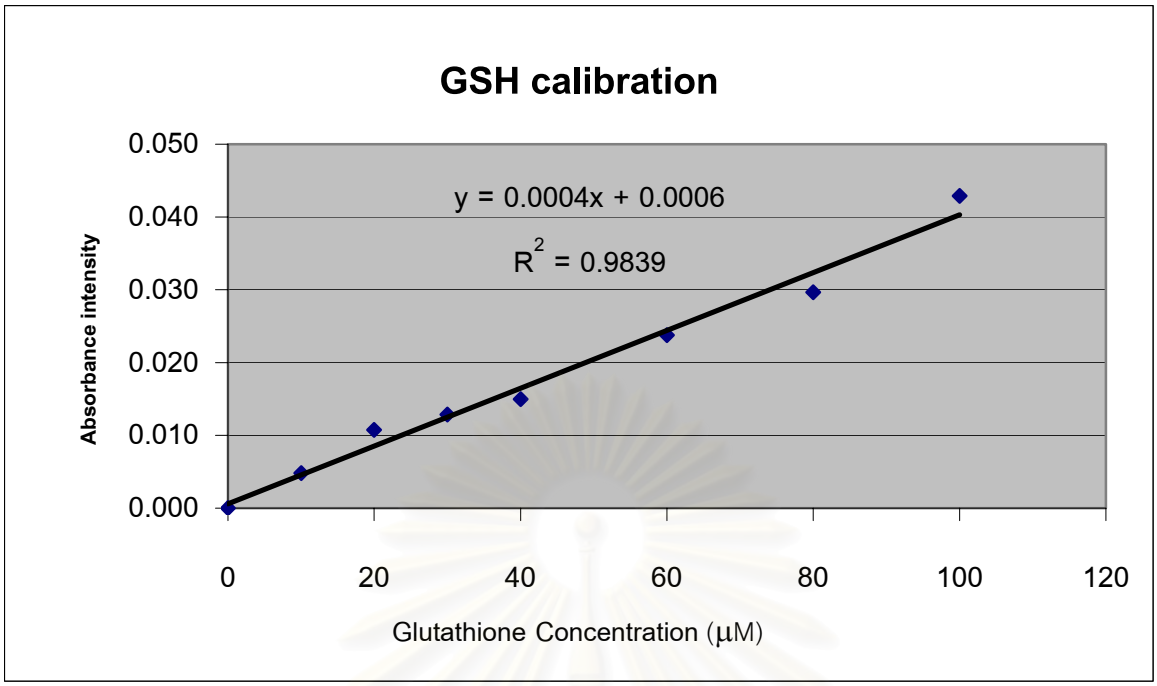


Figure 7 Calibration curve between glutathione concentration and absorbance intensity

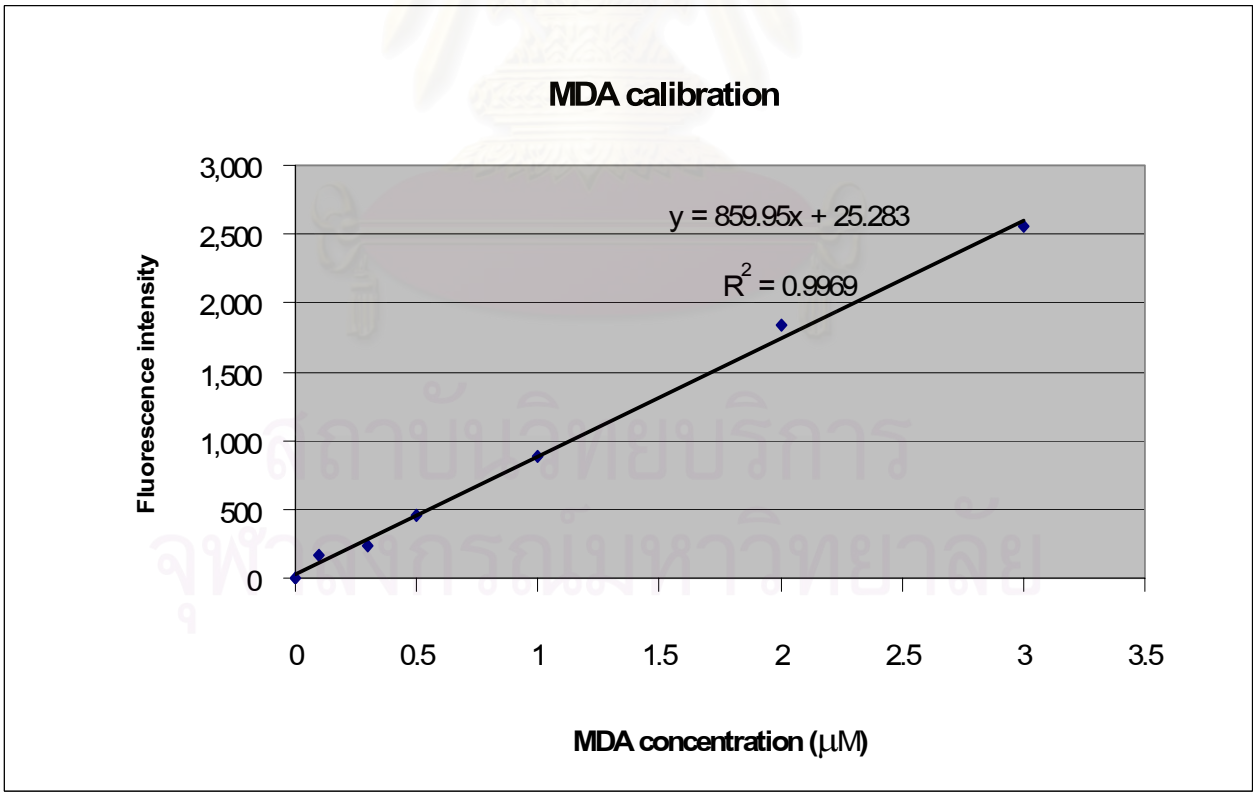


Figure 8 Calibration curve between MDA concentration and fluorescence intensity

Precision and accuracy

Glutathione (GSH)

The precision, accuracy (expressed as the percentage of inaccuracy) of the assay procedure are presented in Table 14 A and Table 14 B, respectively. Percentage of variation coefficient (%CV) of intra-day and inter-day precision of at concentration between 5-90 μM ranged from 4.1-11.5 % and 5.2-14.8%, respectively. The inaccuracy of the assay method was less than 15% for both intra-day and inter-day assay.

Malondialdehyde (MDA)

The precision, accuracy (expressed as the percentage of inaccuracy) of the assay procedure are presented in Table 15 A and Table 15 B respectively. Percentage of variation coefficient (%CV) of intra-day and inter-day precision at concentration between 0.1-3 μM ranged from 4.2-7.2% and 5.2-14.5%, respectively. The inaccuracy of the assay method was less than 20% for both intra-day and inter-day assay.

Recovery

The recovery of GSH and MDA assay are presented in Table 17 and Table 18. The inter-day and intra-day GSH assay recovery ranged from 98.8-102.1% and 95.4-100.5%, respectively. While the intra-day and inter-day recovery of MDA assay ranged from 94.6-105.4 % and 93.3-109.7%, respectively.

Table 14 Precision and accuracy of the assay procedure for GSH

A. Intra-day precision (n = 5)

Target concentration (μM)	Found concentration[a] (μM)	%CV	% inaccuracy[b]
5	5.03 (0.56)	11.5	8.4
50	51.1 (2.86)	5.6	5.4
90	88.9 (3.64)	4.1	3.0

B. Inter-day precision (n = 15)

Target concentration (μM)	Found concentration[a] (μM)	%CV	% inaccuracy[b]
5	4.77 (0.71)	14.8	12.0
50	50.2 (4.20)	8.3	6.9
90	86.1 (4.50)	5.2	5.2

[a] expressed as mean (SD)

[b] $\%\ \text{inaccuracy} = \left| \frac{(\text{Found concentration} - \text{Target concentration})}{\text{Target concentration}} \right| \times 100$

Table 15 Precision and accuracy of the assay procedure for MDA

A. Intra-day precision (n = 5)

Target concentration (μM)	Found concentration[a] (μM)	%CV	% inaccuracy[b]
0.1	0.12 (0.01)	8.5	17.29
0.5	0.46 (0.02)	3.9	8.59
3	2.78 (0.12)	4.2	7.29

B. Inter-day precision (n = 15)

Target concentration (μM)	Found concentration[a] (μM)	%CV	% inaccuracy[b]
0.1	0.12 (0.02)	14.5	17.14
0.5	0.50 (0.04)	7.6	6.86
3	2.83 (0.15)	5.2	6.32

[a] expressed as mean (SD)

[b] $\%\ \text{inaccuracy} = \left| \frac{(\text{Found concentration} - \text{Target concentration})}{\text{Target concentration}} \right| \times 100$

\

Table 16 Recovery of assay procedure for GSH

Target concentration (μM)	Recovery[a]	
	Intra-day[b]	Inter-day[b]
5	100.7 (11.5)	95.4 (14.1)
50	102.1 (3.7)	100.5 (8.4)
90	98.8 (4.0)	95.7 (5.0)

Table 17 Recovery of assay procedure for MDA

Target concentration (μM)	Recovery[a]	
	Intra-day[b]	Inter-day[b]
0.1	100.2 (10.5)	109.7 (12.9)
0.5	105.4 (5.1)	97.7 (5.9)
3	94.6 (2.2)	93.3 (4.3)

[a] $\%\ \text{recovery} = \dfrac{\left|\text{Concentration found after analytical process}\right| \times 100}{\text{Concentration added}}$

[b] expressed as mean (SD)

Distribution and correlation of oxidative status in SLE patients with different severity.

This study was performed in 4 groups (healthy subjects, mild, moderate, and severe SLE patients). The plasma GSH, MDA levels were measured in all groups to study the distribution and comparison of oxidative status in SLE patients with different severity. Almost all SLE patients were female (20 females in mild, 20 females in moderate, and 3 male and 11 females in severe group, respectively). Control group consisted of 3 males and 17 females and characteristic of the participants was shown in Table 18. The oxidative status are shown in Table 19, Figure 9 , Figure 10, Figure 11, and Figure 12, respectively.

Table 18 The characteristics of SLE patients and controlled group

	Control	SLE patients			P value
	(n=20)	Mild (n=20)	Moderate (n=20)	Severe (n=14)	
Age[a]	44.9 ±15.6	37.7±13.2	36.6±10.6	40.1±9.5	0.173
Smoking habits	No smoke	No smoke	No smoke	No smoke	1.00
SLE duration[a]	0	4.5 ±3.3	6.2± 6.1	5.6 ± 3.9	0.515
SLEDAI score[a]	0	3.4 ±1.8	13.3 ±1.3	37.8 ± 8.7	0.0001*
Drug administration					
Prednisolone N, (%)	-	17, (85)	19, (95)	14, (100)	0.227
Prednisolone dose[a], mg/wk	-	86.9 ±110.0	154.9± 89.3	447.5±328.2	0.0001*
Antimalarial (%)	-	13, (65)	13, (65)	9, (64)	0.274
Cq dose[a] , mg/wk	-	400.0±675.7[b]	950.0±817.6[b,c]	428.6 ±716.7[c]	0.044*
HCq dose[a] , mg/wk	-	410.0 ±613.8	260.0±539.4	500.0 ±696.1	0.510
Other Immunosuppressives (%)	-	5, (25)	16, (80)	9,(64)	0.002*
AZA dose[a] , mg/wk	-	35.0 ±107.7	140.0 ±175.9	102.2 ± 162.8	0.093
CYP dose[a] , mg/wk	-	35.0 ±107.7	170.0 ±317.2	203.6 ± 331.4	0.137

Cq = chloroquine, HCq = hydroxychloroquine,

AZA = azathioprine, CYP = cyclophosphamide

* Significant difference p< 0.05

[a] Express as mean ± SD

[b] p = 0.023 (mild vs moderate)

[c] p = 0.049 (moderate vs severe)

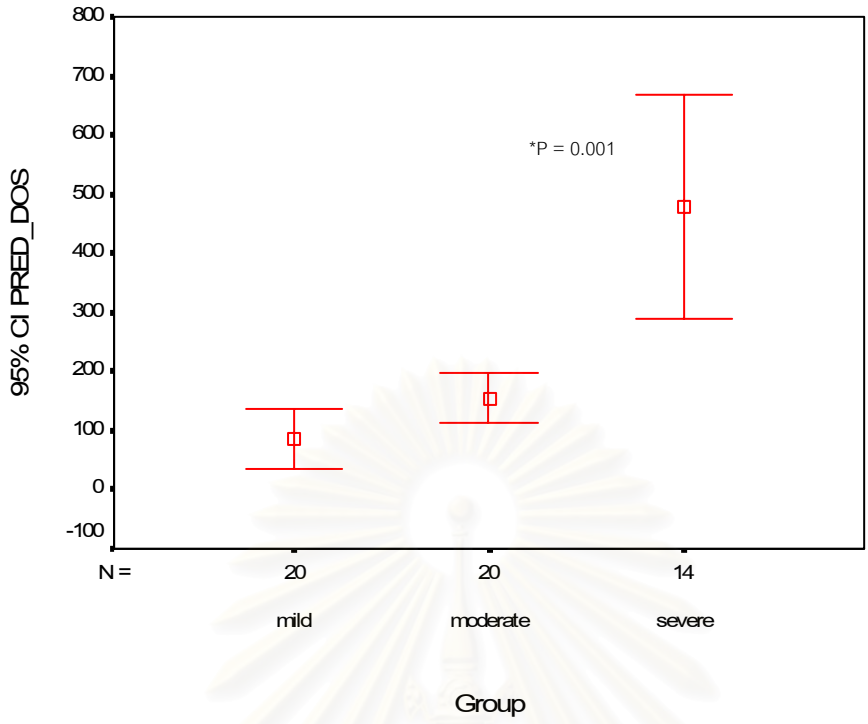


Figure 9 The 95% CI of prednisolone dose (mg/week)

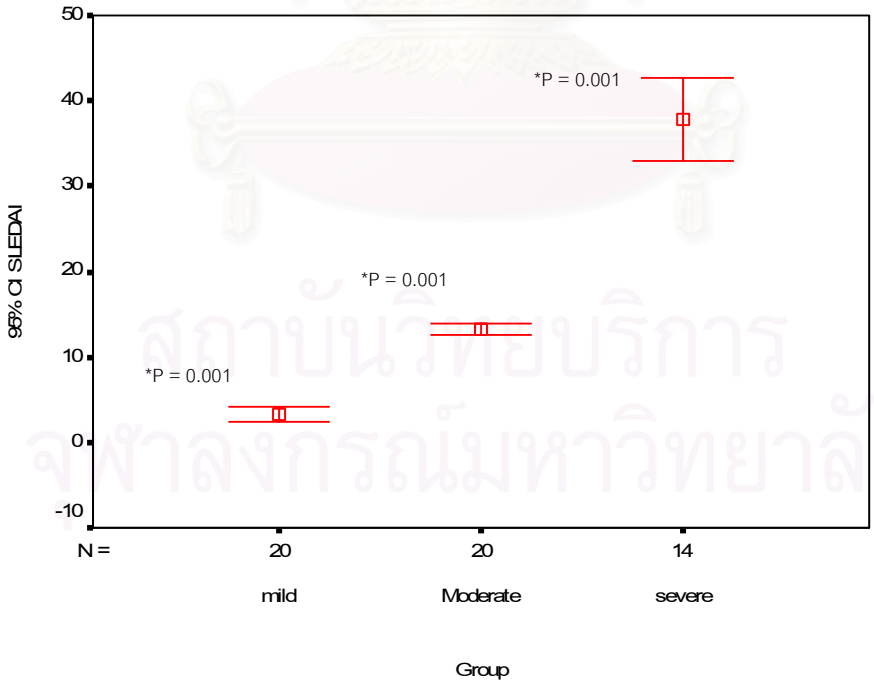
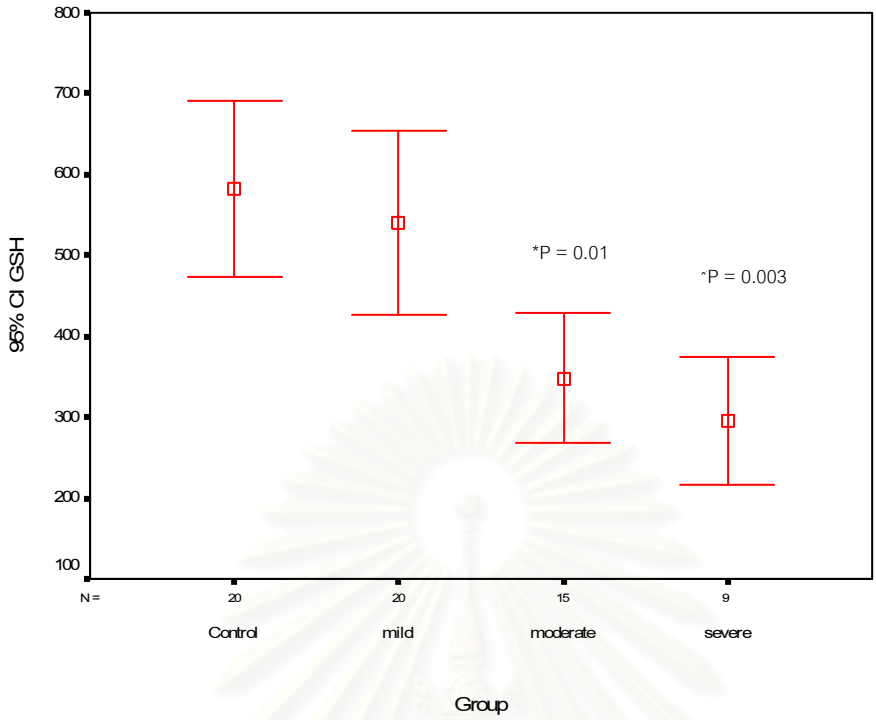


Figure 10 The 95% CI of SLEDAI score

Table 19 Oxidative status parameters in SLE and control patients

Parameters	Mean (SEM)			
	Control (N=20)	Mild SLE (N=20)	Moderate SLE (N=20)	Severe SLE (N=14)
Plasma Glutathione (μM)	582.39 (52.37)	539.503 (54.35)	365.660 (37.80)	312.800 (36.65)
P-value	1.00	0.915	0.01*	0.003*
Plasma MDA (μM)	0.900 (0.118)	0.787 (0.095)	0.681 (0.068)	0.840 (0.216)
P-value	1.00	1.00	1.00	1.00

* Significant different at p< 0.5 level compared with control



* Significant different at p< 0.5 level compared with control

Figure 11 The 95% CI of plasma GSH concentration (μM)

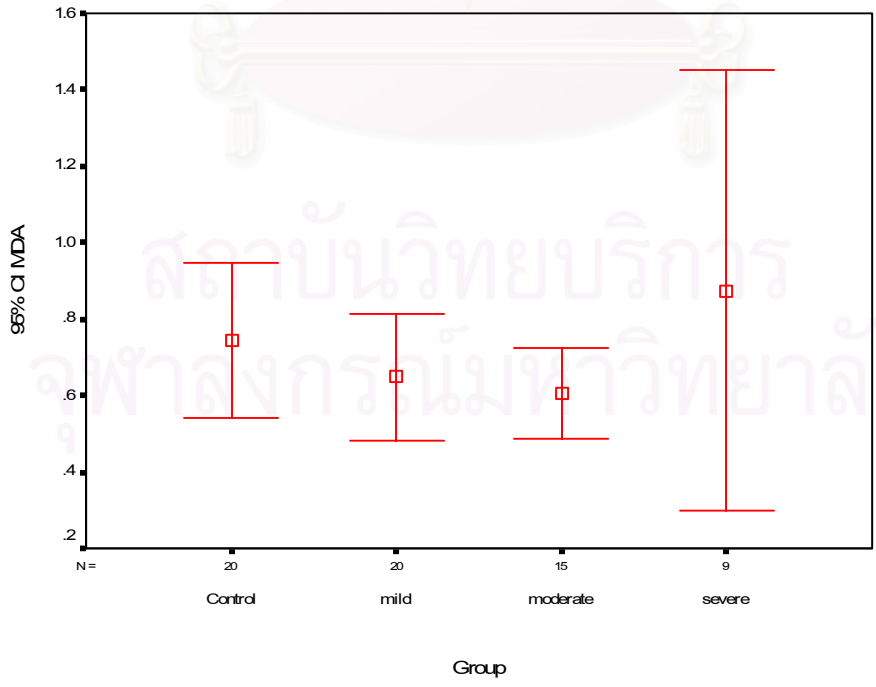


Figure 12 The 95% CI of plasma MDA concentration (μM)

The correlation of plasma glutathione, plasma MDA, prednisolone doses with severity of SLE were studied. Although there was no significant correlation of plasma MDA concentration and SLE severity, the positive relationship was observed (pearson correlation = 0.029, p=0.835) while there was a notice of significant negative correlation of plasma GSH (pearson correlation = -0.427, p=0.001). The regression equation to predict the GSH level based on the severity of SLE disease was as followed;

GSH level (μM) = -117 group severity +638.068 (p= 0.006)

Severity ; 1= mild, 2= moderate, 3 = Severe

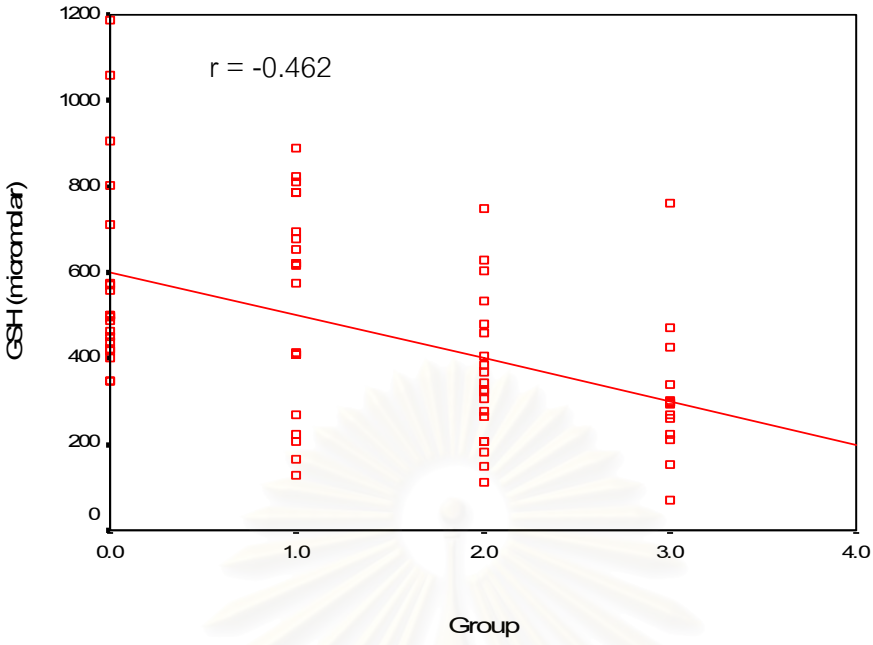
In addition, the correlations of MDA and glutathione levels with SLEDAI score in SLE patients were also evaluated. Significant correlation between SLEDAI and glutathione level was observed (pearson correlation = -0.414 , p<0.001). The MDA level was also related to SLEDAI score, but the correlation coefficient was lower than that of glutathione concentration and no statistically significant difference was observed (pearson correlation = 0.129 , p = 0.351). The linear regression analysis proposed the following equation for prediction of GSH levels using SLEDAI scores.

GSH level (μM) = -6.704 SLEDAI + 463.717 (p= 0.008)

Figure 13 and 14 represent the correlation between plasma GSH concentration and group severity and between plasma GSH concentration SLEDAI were shown in Figure 13 ,14, respectively.

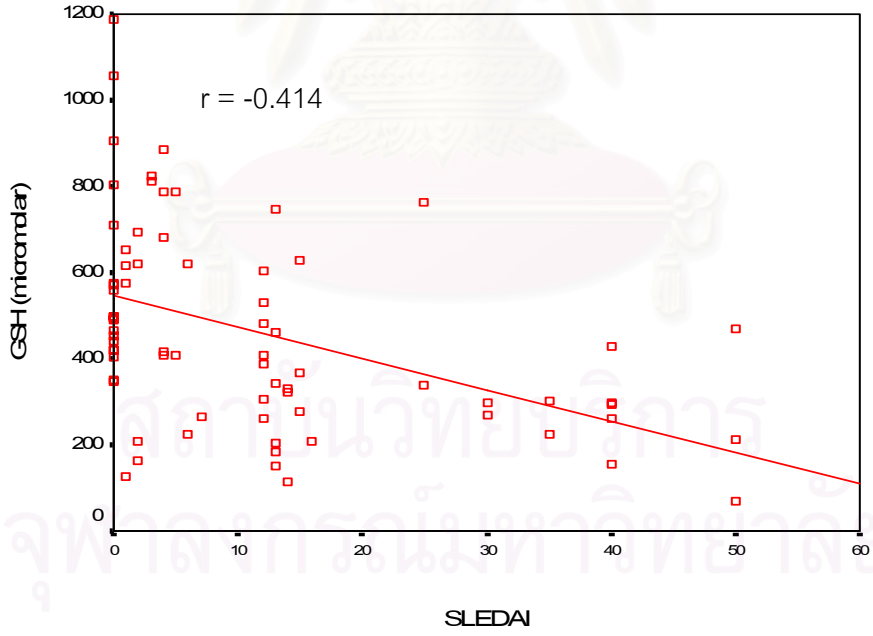
The correlation of prednisolone dose and SLE severity

There were significant correlations between prednisolone dose and SLE severity (also when presented as SLEDAI) as shown in Figure 15 and 16, respectively.



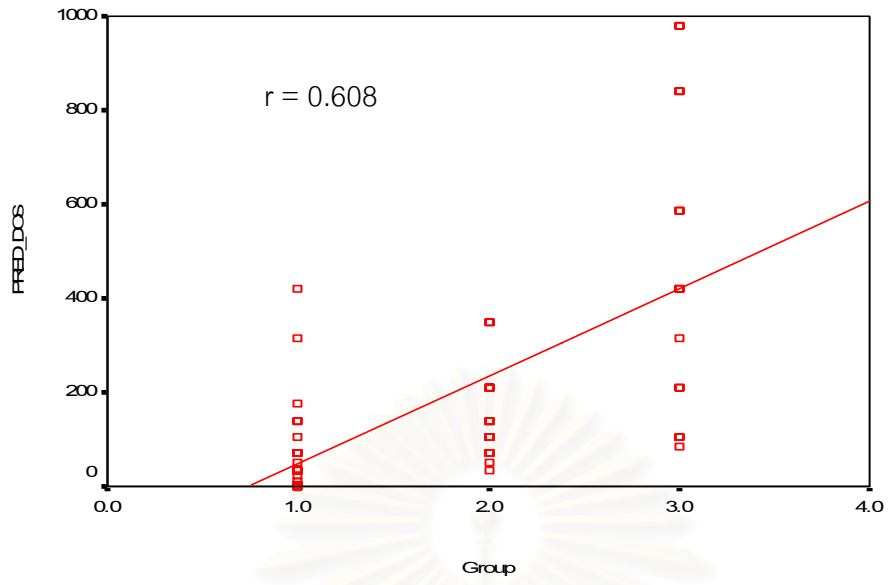
GSH level (μM) = -117 Group severity +638.068 (p= 0.006)

Figure 13 Correlation between GSH and group severity



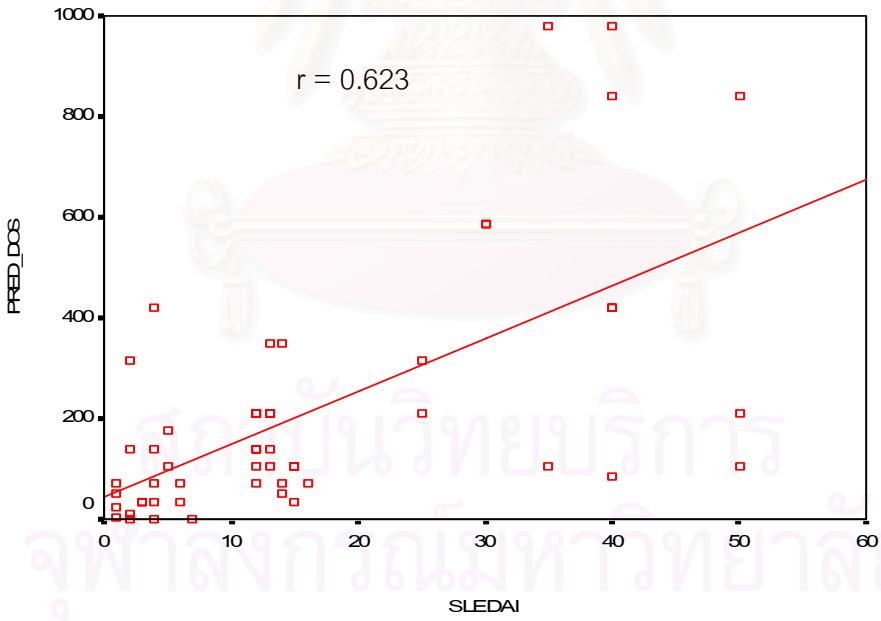
GSH level (μM) = -6.704 SLEDAI + 463.717 (p= 0.008)

Figure 14 Correlation between GSH and SLEDAI



Prednisolone dose (mg/week) = 186.82 Group severity -139.558 (p= 0.0001)

Figure 15 Correlation between prednisolone dose and group severity



Prednisolone dose (mg/week) = 10.509 SLEDAI +45.58 (p= 0.0001)

Figure 16 Correlation between prednisolone dose and SLEDAI

Effect of NAC on lipid peroxidation and GSH in mild SLE patient

Table 20 represent the characteristics of mild SLE patients who were randomed allocation as control and NAC administration, respectively. Patients in 2 groups had almost similar characters except platelet and ALT which were statistically different between NAC group and control group. However, these differences had no significantly effect on GSH or MDA levels.

The statistical difference of plasma GSH between 2 groups was not found. While the significant difference between 2 groups was noted in plasma MDA between 2 groups as presented in the detail of the GSH and MDA concentrations of each patients in Table 21 and 22, respectively. Summary effect of NAC on plasma GSH and MDA in 6 months are illustrated in Table 23 , Figure 17 and 18, respectively. The effect of NAC on red blood cell GSH and red blood cell MDA including the method of determination of GSH and MDA in red blood cell were also written and discussed in Appendix C.

Table 20 Demographic data of SLE patients participated in the study as control group and NAC group at baseline.

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			
Prednisolone containing regimens	18	17	0.500
Hydroxychloroquine containing regimens	13	17	0.273
Azathioprine containing regimens	7	7	0.629
NSAIDs containing regimens	4	4	0.653
Prednisolone +Hydroxychloroquine combination regimens	11	15	1.00
Prednisolone +Azathioprine combination regimens	7	5	1.00
Prednisolone + Hydroxychloroquine +Azathioprine Combination regimens	4	3	1.00
Prednisolone dose (mg/week)	130.75 ± 31.14	118.38 ± 25.52	0.76
Laboratory data			
Hemoglobin (mg/dL, mean ± SD)	11.9 ± 1.17	12.3 ± 1.47	0.314
Hematocrit (mg/dL, mean ± SD)	35.2± 3.57	36.5± 4.31	0.307
SLEDAI score	2.65 ± 1.46	2.15 ± 1.53	0.426
WBC (cell/m^3 , mean ± SD)	5776± 2156	6713± 3957	0.269
Plt (cell/m^3 , mean ± SD)	246905 ± 90894	314900± 81721	0.017*
ESR (h^{-1} , mean ± SD)	34.16 ± 22.8	33.10 ± 24.89	0.851
SCr (mg/dL, mean ± SD)	0.786 ± 0.335	0.760± 0.210	0.891
AST ((IU. , mean ± SD)	36.67 ± 1.15	47.33 ± 27.6	0.347
ALT ((IU. , mean ± SD)	87.75 ± 38,38	36.60 ± 18.40	0.039*

WBC = white blood cell, Plt= platelet, ESR= erythrocyte sedimentation rate, Scr = Serum creatinine, AST= Aspartate aminotransferase, ALT = Alanine amino transferase

* Significant difference at p< 0.05 level

Table 21. The plasma GSH of each patients in 2 groups at baseline and at 6 months

Patient No	Plasma GSH (μM) at base line		Patient No	Plasma GSH (μM) at 6 months	
	Control group	NAC group		Control group	NAC group
1	471.90	692.78	1	483.72	521.39
2	621.78	409.31	2	478.32	948.46
3	484.59	516.73	3	545.53	580.3
4	409.94	562.62	4	473.67	247.45
5	570.20	313.58	5	480.44	862.26
6	452.71	717.06	6	418.57	1234.3
7	356.93	409.93	7	3625.45	893.39
8	512.0	491.12	8	269.19	503.7
9	340.29	498.33	9	786.66	370.27
10	1964.87	1970.30	10	1109.6	325.33
11	898.59	509.87	11	342.29	2918.39
12	784.23	766.04	12	1348.4	238.63
13	786.90	473.83	13	219.76	338.32
14	533.46	700.98	14	879.97	460.12
15	269.99	219.28	15	839.61	318.74
16	82.08	230.59	16	1070.31	517.81
17	499.51	2018.45	17	813.73	613.46
18	350.56	438.72	18	1063.76	353.28
19	434.62	1059.60	19	766.64	794.52
20	588.67	470.60	20	761.83	1005.85
Mean + SD	**570.69 ± 377.55**	**673.49±491.31**	**Mean ± SD**	**838.87±738.64**	**702.30±592.97**
P value	**0.932**		**P value**	**0.250**	

Table 22. The plasma MDA of each patients in 2 groups at baseline and at 6 months

Patient No	Plasma MDA (μM) at base line		Patient No	Plasma MDA (μM) at 6 months	
	Control group	NAC group		Control group	NAC group
1	0.265	0.583	1	0.214	0.214
2	0.981	0.980	2	0.239	0.239
3	0.347	1.007	3	0.844	0.844
4	0.235	0.898	4	0.762	0.762
5	0.619	0.631	5	0.308	0.308
6	0.522	0.697	6	0.256	0.256
7	0.265	0.621	7	0.221	0.221
8	0.331	0.217	8	0.331	0.331
9	0.592	0.344	9	0.420	0.420
10	0.797	0.314	10	0.290	0.290
11	0.235	0.292	11	0.268	0.268
12	0.361	0.189	12	0.329	0.329
13	0.490	0.346	13	0.318	0.318
14	0.550	0.868	14	0.135	0.135
15	0.341	0.327	15	0.233	0.233
16	0.464	0.651	16	0.367	0.367
17	0.789	0.280	17	0.358	0.358
18	0.216	0.450	18	0.530	0.530
19	0.141	0.189	19	0.419	0.419
20	0.239	0.246	20	0.233	0.233
Mean ± SD	**0.439± 0.227**	**0.507± 0.274**	**Mean ± SD**	**0.472± 0.286**	**0.354± 0.178**
P value	**0.655**		**P value**	**0.138**	

Table 23 Effect of NAC on plasma GSH and plasma MDA concentrations at 6 months

Parameters (Mean± SEM)	Group	Baseline	6 months	P value
MDA (μM)	Control	0.439 ± 0.057	0.472 ± 0.076	0.875
	NAC	0.507 ± 0.068	0.354 ± 0.040	0.023*
GSH (μM)	Control	574.56 ± 83.98	838.87 ± 161.84	0.174
	NAC	673.48± 70.78	702..30 ± 132.59	0.702

*Significant difference at 0.05 level

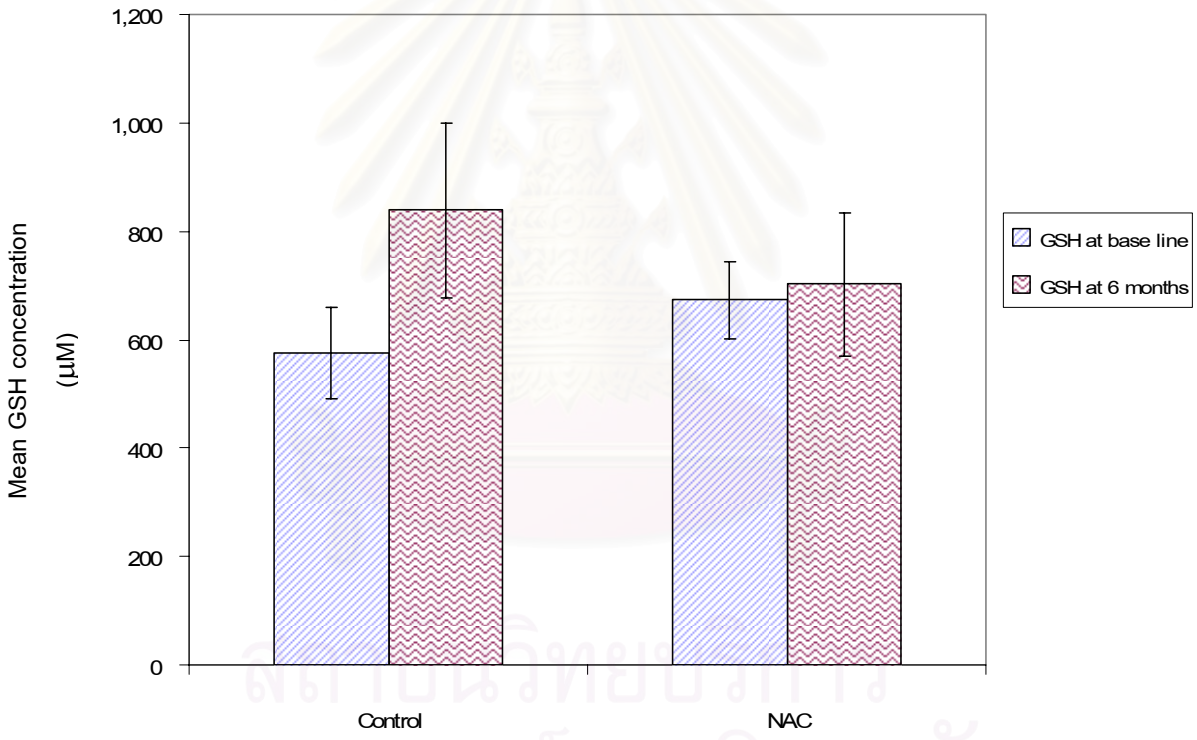


Figure 17 Effect of NAC on mean GSH concentration (μM)

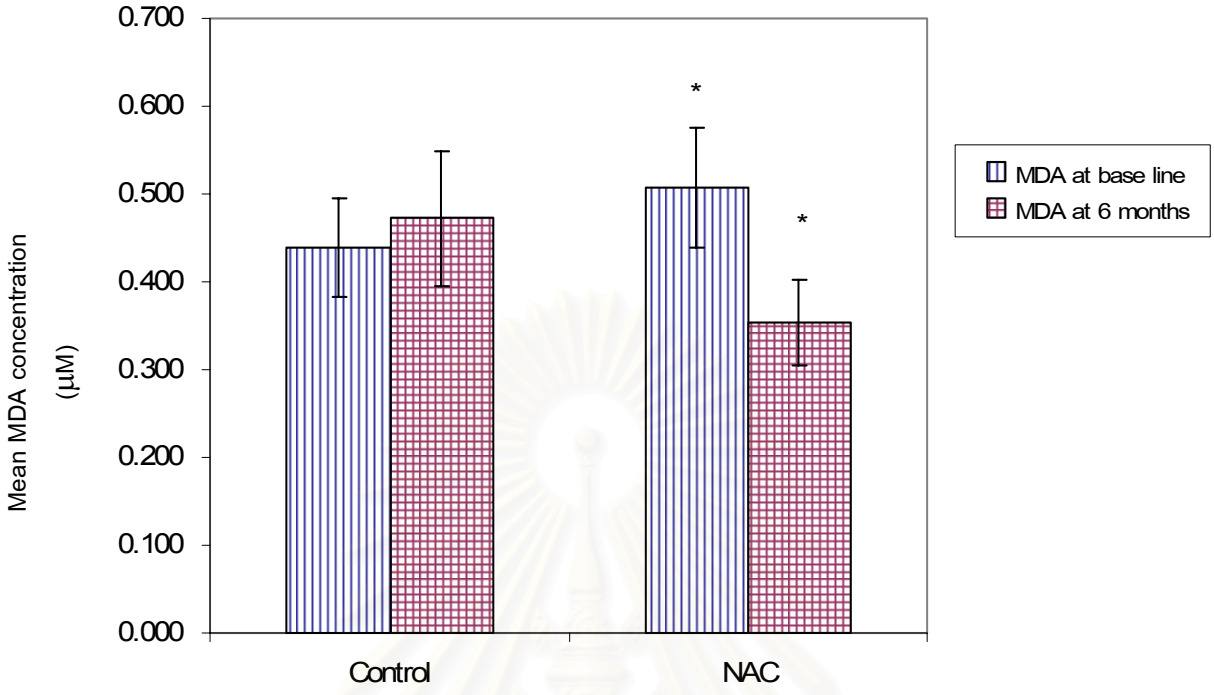


Figure 18 Effect of NAC on mean MDA concentration (µM)

* Significant difference at 0.05 level (p = 0.023)

Effect of NAC on prednisolone dose and SLEDAI score in mild SLE patients

The individual prednisolone dosage of SLE patients in each group was presented in Table 24. There was statistically difference in proportion of SLE patients who could taper and could not taper prednisolone. All patients in NAC group could tapper prednisolone, while only 13 patients in control group could decrease prednisolone dose. The chi-square test was illustrated in Table 25 and Table 26, respectively. Although significant different of prednisolone dose between baseline and at 6 month therapy was found in both groups. However, that significant was only found in NAC group when classified by SLEDAI score as less than 4 and more than or equal to 4 (as presented in Table 27 ($p<0.05$)). The SLEDAI score of each patient in 2 groups (control and NAC group) were presented in Table 28. SLEDAI score in NAC group was significant difference from control group as shown in Figure 19. The significant difference between subject in 2 groups was found ($p< 0.05$) by Analysis of Variance (ANOVA) test as shown in appendix A. Consider about relapse, one patient in control groups was relapsed during tapper prednisolone dose while no one in NAC group was relapsed. Five patients who received NAC were withdrawn from the study; one was inconvenient to have regularly followed up, one subject had allergy of excipient in NAC preparation, two subjects had severe headache, and one subject had lost follow up. The replacement of patients in NAC group was performed by random sampling.

Table 24. The prednisolone dose (mg/week) of each patients in 2 groups at baseline and at 6 months

Patient No	Prednisolone (mg/week) at base line		Patient No	Prednisolone (mg/week) at 6 months	
	Control group	NAC group		Control group	NAC group
1	35.0	17.5	1	17.5	0.176
2	140.0	105.0	2	70.0	0.197
3	315.0	140.0	3	105.0	0.695
4	35.0	140.0	4	17.5	0.628
5	0.0	420.0	5	140.0	0.254
6	140.0	350.0	6	35.0	0.211
7	105.0	0.00	7	70.0	0.182
8	17.5	105.0	8	0.0	0.273
9	315.0	270.0	9	140.0	0.346
10	35.0	140.0	10	7.5	0.239
11	315.0	0.0	11	105.0	0.221
12	35.0	17.5	12	35.0	0.271
13	17.5	70.0	13	17.5	0.262
14	0.0	67.5	14	0.0	0.111
15	315.0	35.0	15	35.0	0.192
16	280.0	70.0	16	105.0	0.302
17	25.0	0.0	17	25.0	0.295
18	420.0	140.0	18	35.0	0.437
19	35.0	140.0	19	70.0	0.345
20	35.0	140.0	20	35.0	0.192
Mean + SEM	**130.75+ 31.14**	**118.38+ 25.51**	**Mean + SEM**	**53.25+ 10.00**	**47.52+ 11.37**
P value	**0.760**		**P value**	**0.707**	

P= 0.012

P= 0.002

Table 25 Number of SLE patients who can tapper or cannot tapper prednisolone

		GROUP		Total
		Control (n=20)	NAC (n=20)	(n=40)
PREDTAPE *	No tapper	7	0	7
	tapper	13	20	33

* p = 0.008

* Significant difference at 0.05 level

Table 26 Chi-square test of prednisolone tappering in control group versus NAC group

	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		
N of Valid Cases	40				

* Significant difference at 0.05 level

Df = degree of freedom

Asymp. Sig = asymptotic significance

Table 27 Effect of NAC on prednisolone dose at 6 months

Parameters (Mean± SEM)	**Group**	**N**	**Baseline**	**6 months**	**P value**
Prednisolone dose of all subjects in both group	Control	20	130.75 ± 31.14	53.25 ± 10.00	0.012*
	NAC	20	118.38 ± 25.51	47.52 ± 11.37	0.002*
Prednisolone dose of subjects who had SLEDAI ≥ 4 in both group	Control	9	172.08 ± 68.29	68.83 ± 29.37	0.124
	NAC	6	150.55 ± 70.78	59.16 ± 37.21	0.014*
Prednisolone dose of subjects who had SLEDAI less than 4 in both group	Control	11	141.60 ± 42.69	51.32 ± 15.47	0.274
	NAC	14	97.50 ± 17.57	38.39 ± 10.31	0.0001*

* Significant difference at 0.05 level

Table 28. The SLEDAI score of each patients in 2 groups at baseline and at 6 months

Patient No	SLEDAI score at base line		Patient No	SLEDAI score at 6 months	
	Control group	NAC group		Control group	NAC group
1	1	1	1	0	0
2	1	1	2	2	3
3	4	2	3	2	0
4	4	1	4	4	1
5	1	5	5	0	4
6	4	4	6	3	2
7	4	1	7	0	0
8	0	3	8	0	0
9	4	2	9	0	1
10	2	1	10	2	0
11	2	1	11	0	0
12	4	2	12	4	0
13	4	4	13	1	1
14	1	4	14	0	0
15	4	4	15	2	2
16	4	4	16	2	3
17	4	0	17	4	0
18	2	0	18	2	0
19	2	1	19	2	0
20	2	2	20	0	2
Mean + SEM	**2.65+ 1.46**	**2.15+ 1.53**	**Mean + SD**	**1.50+ 1.47**	**0.95+ 1.28**
P value	**0.30**		**P value**	**0.24**	

P= 0.003

P= 0.001

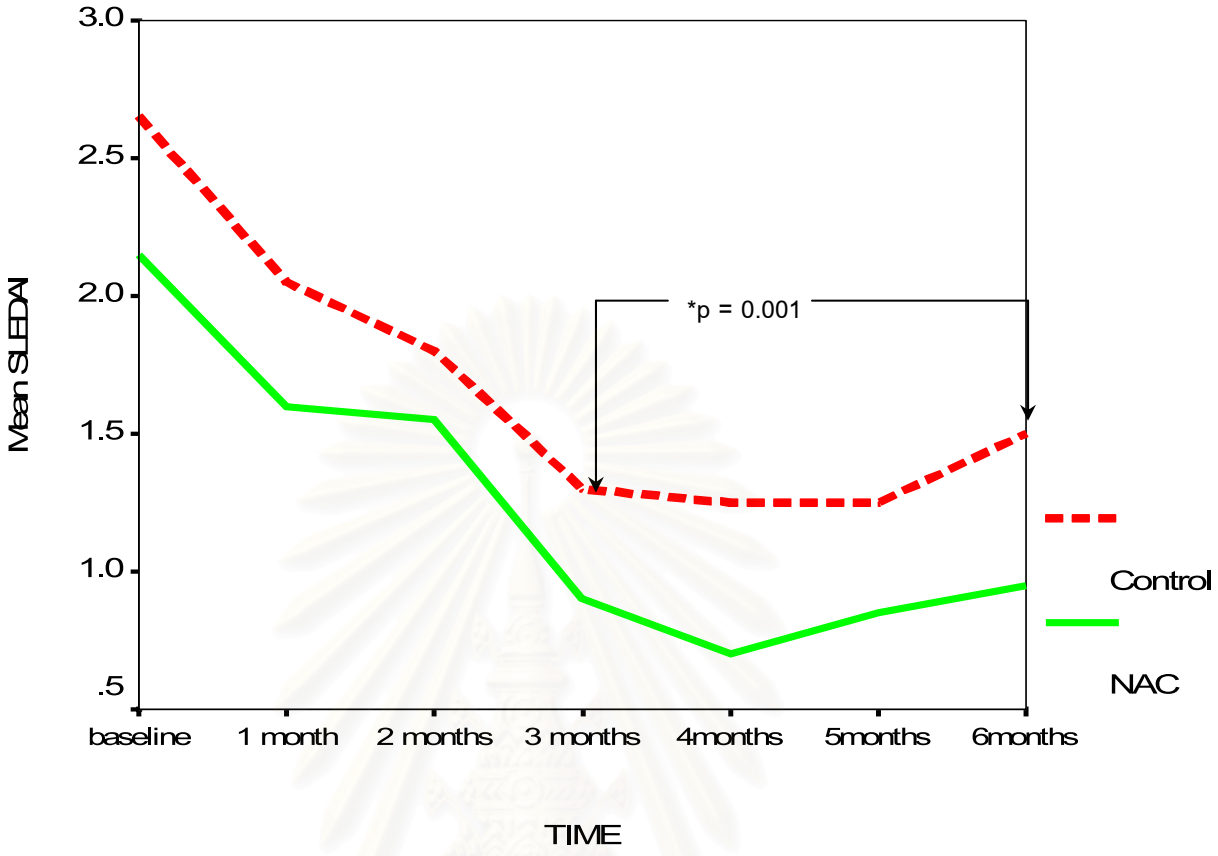


Figure 19 The mean SLEDAI at each month during the 6 months study period.

* significant different ($p<0.05$) between control and NAC group since 3 months of study

Clinical outcome and adverse effect of NAC

According to random interviewing patients who received NAC, four patients informed that they felt better, fresher, and had better skin appearance as compare to before starting NAC. The blood test results and other clinical symptoms such as decrease in hair falling, no new rash appeared, no fatigue, and no joint pain also showed better clinical outcomes. However, there were three patients who might have side effect caused by NAC. Two patients had severe headache which the symptom disappeared when the drug was discontinued. One patient might allergic to the excipient of NAC with rash symptom, because she used to administer another form of NAC (powers) without side effects.

CHAPTER V
DISCUSSION AND RECOMMENDATION

DISCUSSION

Epidemiology and drug's pattern study

From local prevalence study, the number of SLE patients increased from the year 2000 to 2006. The reason for increasing the number of SLE patients might due to the improvement of diagnostic method and the more sensitive evaluation criteria based on the current criteria of ACR. However, this increasing circumstance is a cautious sign for healthcare professionals to concern about this disease.

The drug therapy pattern of SLE at Ramathibodi hospital was similar to international guideline for treatment of SLE.[104-106]. Prednisolone has also been the main core drug therapy, however the exact dose for various severity of SLE has not been standardized. According to systemic side effect of high dose prednisolone, therefore, selection of other immunosuppressive agents for mild to moderate severity is a good strategy to taper steroids usage. In this center, combination therapy with steroids pattern have been the most common choice. Four main drugs were selected to compare the therapeutic outcome among different disease severity; prednisolone, choroquine, azathioprine and hydroxychloroquine. Prednisolone containing regimens gave satisfied therapeutic outcome. The most 2-drugs combinations which were frequent prescribed were prednisolone with hydroxychloroquine, prednisolone with azathioprine, prednisolone with chloroquine, respectively, while the three combination regimen which was frequently used was prednisolone plus azathioprine and chloroquine. However, because of retrospective method, the exact conclusion that prednisolone containing regimens are the most effective should not be implemented. The severity of SLE is not significant factor which effects on the ratio of patients who use and not use prednisolone containing regimens since prednisolone containing regimens were used in SLE patients with any severity, only the dosages were different. The more severity, the more often that higher steroid dosage

will be prescribed. The recent study also supported the role of prescribing high dose of steroids. [107] The most concern during steroids use is its side effect which also occurs at Ramathibodi hospital, however it can be managed by gradually tapered down the dosage of steroids.

Chloroquine and azathioprine were the other two main drugs which were given in satisfied therapeutic out comes in SLE patients. The percentage of cyclophosphamide usage tended to increase in more severity (moderate and severe cases). More SLE patients with moderate and severe level frequently received cyclophosphamide than mild severity patients. This results agreed with previous clinical trials of cyclophosphamide which showed its effectiveness in the treatment of lupus nephritis (LN) patients with are classified as moderate severity of SLE.[108-110]. Hydroxychloroquine is an antimalarial drug that recommended for SLE treatment. The interests in using this drug in SLE patients and the investigation of its effectiveness has been increased. Moreover, the recommendation of measuring hydroxychloroquine level was proposed to monitor its effectiveness in SLE patients since the association of subtherapeutic hydroxychloroquine level and exacerbation of SLE was detected.[111-117]. Currently, recommendation of using hydroxychloroquine was proposed even when patients were in remission to prevent SLE flare or relapsed.

In severe SLE patients, it was found that prednisolone containing regimens give significantly different proportion of patients in terms of better therapeutic outcomes. In contrast, azathioprine and chloroquine containing regimens gave significant higher improvement than those in non-received these regimens group. From the results, it seems that azathioprine and chloroquine may give satisfied respond in severe SLE patients. This finding is in agreement with those previous studies which reported that azathioprine and chloroquine could be used for maintenance therapy in this group of patients, besides, another advantage of these two drugs is their safety to use in pregnancy or children.[105, 118-124] Another point of view, SLE patients who had already been in remission stage were more frequently received azathioprine, choroquine or hydroxychloroquine for maintenance therapy and prevent relapse as previously mentioned.

According to the association results of SLE duration and the frequency use of drugs, when concerning of prednisolone, azathioprine, cyclophosphamide, chloroquine and hydroxychloroquine, there was only significant association between SLE duration and the frequency of using azathioprine, while the rests are given non significant results. This may be implied that, azathioprine is frequently used in early onset of SLE, if the symptoms are more severe. This information is agreed with clinical trials of using azathioprine which has been frequently prescribed in patients who were hospitalized with severe renal involvement (especially in lupus nephritis).[105, 125-133]

Although using of bioactive agents such as anti-CD4 receptors have been recommended in SLE patients who were resisted to the conventional therapy, the frequency of use is still low, this may due to the high cost of the bioactive agents.

The limitation of this study is lacking strong evidence to confirm the efficacy of each drug in treatment of SLE patients because it did not design as clinical trial. However, some results are agreed with the guideline or treatment recommendation (which come from clinical trials).[22, 104, 119, 134, 135] Since, the objective of this study was only to observe the pattern of regimens in the treatment of systemic lupus erythematosus and try to correlate it with clinical outcomes. Another point of the weakness of this study is the cross-sectional design; therefore, the results cannot be implied if the time is changed. For example, about the drug pattern study and clinical outcome found that SLE patients who not received prednisolone had more proportion (percentage) of remission than patients who received prednisolone. However, the started time is not the same. Some patients previously received prednisolone and can stopped taking in the study periods. Therefore, it cannot exactly conclude that prednisolone is not effective even the proportion of remission was less than the patients who did not received prednisolone. Well-controlled clinical trials are the importance supporting for evaluation the effective of using drug regimens. However, because the aim of this study was only to explore the trend of drug using regimens and try to relate with therapeutic outcomes, the information from this study have to use with caution before application to clinical practice. The comparison with previously clinical trials is necessary.

There are many parameters which are used to evaluate the severity of SLE such as SLEDAI 2k, MEXSLEDAI, SLEDAI, BILAG, LAI, ECLAM, SLAM. However, in this study, only SLEDAI was used because it is the most common standard parameter used to evaluate SLE severity, it has been validated for a long time and many centers use it as the routine parameter for monitoring because it was easy to use, only 24 items of information required. Other parameters contain more items leading take more time to evaluate.[100] Although SLEDAI is most commonly use in many centers, but it has a limitation when the laboratory facility is not available. Recent researchers have tried to develop more accurate, precise and simplify tools for evaluation of SLE severity which provided more usefulness in clinical trials.

In lupus congress 2007 conference, the use of standardized monitoring tools in SLE clinical trials are promised after the year 2007.

Validation method of glutathione and MDA analysis

The analysis method of glutathione and MDA were validated. According to the results, both methods showed acceptable specificity, accuracy, precision and recovery. In term of specificity, no change of the absorbance was found when the blank sample was expressed. The percentages of inaccuracies were less than 20%. The acceptable precisions, which received in terms of coefficient of variation, were less than 15%. The linearity of glutathione concentration of 0-100 μM and intensity, MDA concentration of 0-3 μM and intensity were obtained with satisfied correlation coefficient (r). The percentage of recovery was more than 80%. Thus, both methods were appropriate for analysis of glutathione and, MDA in plasma sample. Furthermore, the samples kept frozen at -20 °C were stable for at least 2 weeks.[136] and the storage time of the samples obtained for all patients was not longer than the stability study. This would ensure the assay results. The reasons that selection of spectrophotometer for the measurement of MDA and GSH level are as followed: first, it does not take long time to analyze; second, the use of the instrument does not need high analytical skill as compared to HPLC or LCMS. Therefore, new workers or new members who have less experience of the analytical skill can take the responsibility to this task. Third,

this method has been used in many analytical setting. However, other analytical methods could be developed for more accurate results.

Distribution and correlation of oxidative status in SLE patients with different severity

Comparision of prednisolone, immunosuppressives and antimalarial doses versus SLE severity.

From the results, the more severity of SLE, the higher prednisolone dose was administered. However, due to the concern about side effects of high prednisolone dosage which involve metabolic and many organs system such as bone, ophthalmic and, GI [137], several physicians prefer to add second and/or third drugs (steroid sparing agents) rather than increasing the steroid dosage. Focus on immunosuppressive agents and antimalarials doses, non-significant association between dose of azathioprine or cyclophospharmide versus SLE severity were observed. While there was a significant association between chloroquine dose and severity ($p=0.044$). Its dosage tends to be higher In moderate SLE patients. Focus on moderate SLE patients, the number of SLE patient who received chloroquine quite higher than hydroxychloroquine (12 vs 4, data not shown). This may due to the side effect of chloroquine which cause retinal maculapathy therefore its dose in severe SLE patients was reduced in severe SLE patients.

Distribution and correlation of oxidative status in SLE patients with different severity

Base on the evidence that imbalance of oxidative status involves in the pathology of systemic lupus erythematosus,[26, 98, 102, 138-140] this study was performed to determine the correlation of oxidative status parameters and the degree of severity of SLE disease. From the results, the value of MDA and GSH varied widely. The correlation between severity and MDA level and correlation between severity and GSH level were observed. However, because the nature of SLE diseases has multiple factorials, the correlation coefficients of these 2 parameters (GSH and MDA) were low. The other factors such as duration of SLE, prednisolone dosage should be considered. Larger samples and longer follow up periods

will be needed. Although this study did not show the significant relationship between MDA and severity of the disease, MDA level was higher in more severity SLE patients. This trend is in agreement with previous study which showed the correlation of MDA level and SLEDAI scores,[26] with Pearson's correlation = 0.40. However, this study did found significant correlation at p= 0.05 level between MDA and SLEDAI score. The reason for finding lower correlation in this study may due to the effect of prednisolone on lipid peroxidation, since the recruited patients in previous study did not received prednisonlone.

There were four severe SLE patients who had low GSH levels and then were died because of complication. This supported the important role of GSH to be set as a marker of oxidative organ damage.

Gluthatione precursor's supplementation should be considered in severe SLE patients to improve oxidative status. However, more research about this propose should be performed and other factors should also be included into the analysis such as other underlining diseases, co-administration drugs, etc.

Effect of NAC on lipid peroxidation and GSH in mild SLE patients

According to the results, administration of 1800 mg NAC as adjunctive therapy did not give significant different in GSH level. However, the MDA level of NAC group was significantly lower than control group after six months of therapy. The result was similar to the phase I study about the effect of NAC as a chemopreventive in patients who suffered from malignancy.[34] No correlation between NAC concentration and percentage of change of GSH in peripheral blood lymphocyte in malignant patients. The effect of NAC as glutathione precursor has prolonged for a short time and this positive effect occurred only in some patients.[34]. The pitfall of this study may result from part 2.in this study since the GSH level of mild SLE patients did not significant different from control. Therefore, the significant effect of increase of GSH was not observed when NAC were administered in mild SLE patients. Another reason may be due to pro-oxidant effect of NAC which has been reported to be found from *in vitro* study [141]. High dose of NAC may cause higher free radicals and make lower GSH level which were lower than control group.

However, significant anti-oxidant effect [5, 142, 143] was also found as MDA level was lower in NAC group at 6 months.

Focus on mechanism of action of NAC which may be able to explain the results, it reveals that even though deacetylation of NAC releases cysteine which is the primary agent for glutathione synthesis. However, the synthesis process of GSH does not only require cysteine, the two more amino acids; glycine and glutamine are also required in the later processes. In addition, the activity of GSH synthesis enzymes may be an intrinsic factor. Therefore, GSH level did not increase significantly after co-administration with NAC. In contrast, this MDA synthesis process, require only the step of lipid peroxidation of fatty acid and this lipid peroxidation process is inhibited by NAC, therefore, a significant decrease in plasma MDA level could be found. This simple explanation might more clarified by Figure 20. Polymorphism issue of glutathione synthase enzyme in SLE patients which made the variation of GSH levels were also established. [144-146]

Effect of NAC on prednisolone dose and SLEDAI score in mild SLE patients

All patients in NAC group could tapper prednisolone dose. While only 13 out of 20 patients in control group who could decrease prednisolone dose. The result showed statistically significant difference in proportion of patients who could tapper prednisolone between NAC group and control group. (p=0.008).

Focus on the SLEADAI score, the effect of NAC on SLEDAI score seemed to be found since third months of NAC's administration. It may be implied that the initial effect of NAC that lowering MDA had seen since about three months of its administration.

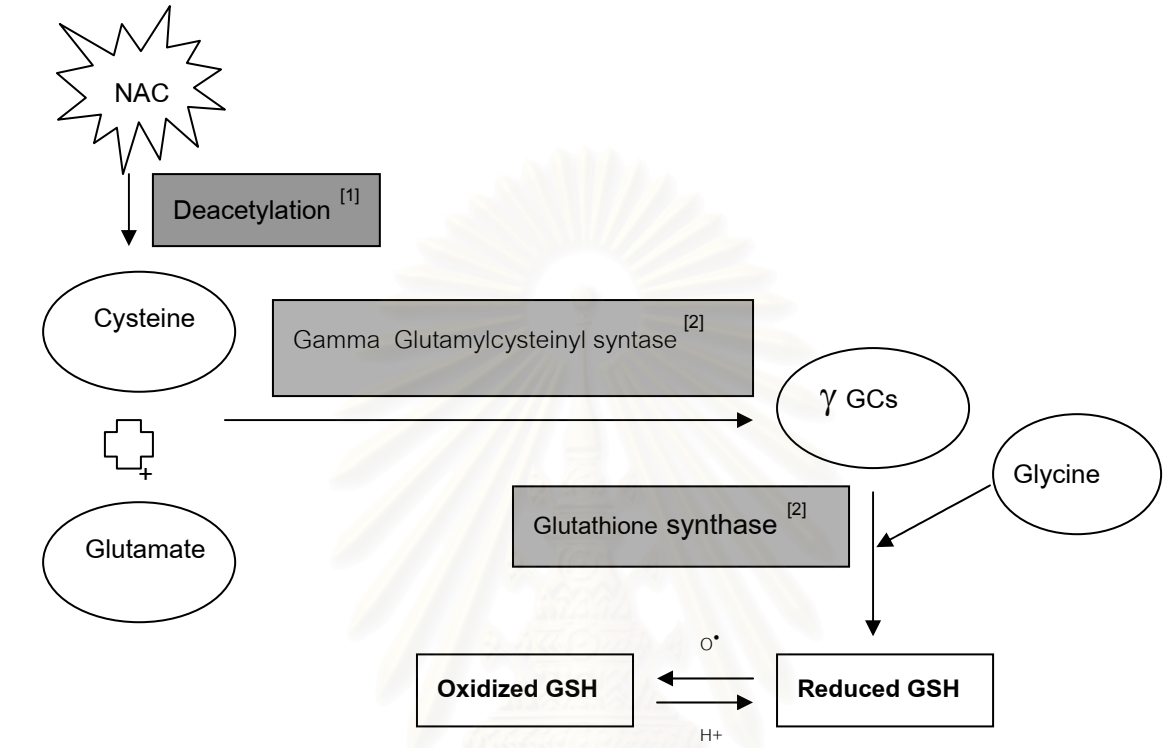
This study had some limitation. First, only one fixed dose of NAC was administered. Therefore, the results showed only the effect of 1800 mg NAC. Further studies should be performed in another dose of NAC. In fact, patients should be recruited into the study since their first visit and baseline data should be taken while the patient had not been treated with prednisolone or other drugs. However, in this study

which was performed in tertiary care center, it was difficult to recruit new case of patient who had not been treated with any drugs. Even this study tried to match cases by age, the truly matched cases between 2 groups could not be performed. Further match case study should be repeated for reducing as much as confounding factors as possible.

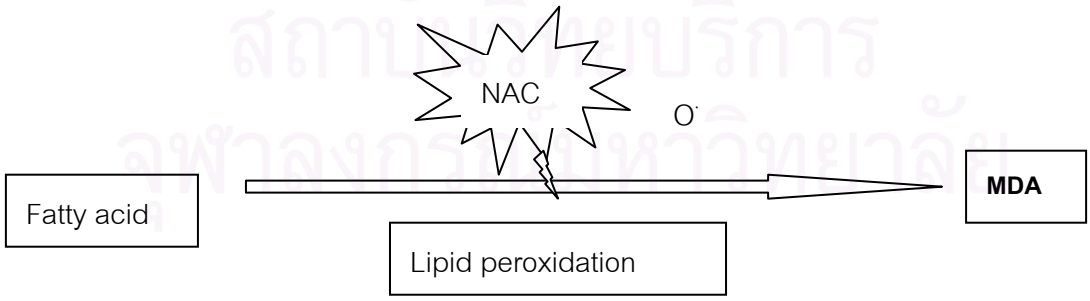
Another limitation is about severity of SLE. In this study, only mild SLE patients were recruited. Evaluation of the effect of NAC in moderate or severe SLE patients should be performed; more distinctive effect of NAC may be concluded.

No standard tool was used in the process of interviewing the SLE patients. However, at least, the information obtained from this study may enhance investigators to perform a better designed study to find out the effect of NAC on other dimension of out comes, for example evaluating the quality of life by build up a series of questionnaire.

In conclusion, administration of 1800 mg NAC may help SLE patients who were classified as minor symptoms to taper prednisolone dose to reduce undesired side effects. The MDA level was significantly lower after using NAC for 6 months. The quality of life of several patients seem to be better, no serious side effect was found.



(a)



(b)

Figure 20 Effect of NAC on the process of GSH and MDA synthesis

RECOMMENDATION

Further study is recommended as the following issues

Larger number of subjects, more frequent observation and longer period of follow up should be performed.

Different doses of NAC should be implemented parallel with the pharmacokinetic of NAC in the patients.

Expansion of NAC to moderate and severe SLE should be established.

Expansions of duration of NAC administration are recommended to evaluate long term effect of NAC.

Measurement of other biological substance, such as the activity of GSH synthesis enzymes (glutathione peroxidase, glutathione synthase) should be evaluated to better understanding the mechanism of NAC.

Further investigation about the relationship of GSH or MDA in plasma and red blood cell are required to find out the reason of inconsistent relationship.

Some other oxidative stress markers may be used to confirm the effect of NAC in reducing oxidative stress of SLE patients.

The cost effectiveness of NAC administration as adjunctive therapy in SLE patients should also be evaluated in the later step if the co-administration of NAC has been confirmed to cause some benefit in SLE patients.

CHAPTER VI

CONCLUSION

From the result of three parts it can conclude that

1. There have been an increase in the prevalence of SLE during 7 years (2000 – 2006) from 267.18 to 552.64 per 100,000 patients. The increasing may be also due to the development of diagnostic procedure and criteria.

2. Prednisolone is still the major drug in the treatment of SLE, while the combinations with others immunosuppressants are also increased. The drug therapy pattern at Ramathibodi hospital was similar to international guideline for SLE treatment. Combination drug regimens was frequently used. The two drug regimens which was commonly used in Ramathibodi hospital were prednisolone plus hydroxychloroquine, prednisolone plus azathioprine and prednisolone plus chloroquine. The commonly three combination drug regimens was prednisolone plus azathioprine plus hydroxychlorquine. The prednisolone containing regimens gave more significantly satisfied outcome when compared to non prednisolone containing regimens.

3. The significant correlation between GSH concentration and severity of SLE was observed. While the significant correlation of MDA concentration with different severity was not found.

3.1 The regression equation of correlation of GSH and severity was proposed as the following:

GSH level (μM) = -117 Group severity +638.068 (p= 0.006)

3.2 The regression equation of correlation of GSH and SLEDAI scores was proposed as the following:

GSH level (μM) = -6.704 SLEDAI + 463.717 (p= 0.008)

3.3 The regression equation of correlation of prednisolone dose and severity was proposed as the following:

Prednisolone dose (mg/week) = 186.82 Group severity -139.558 (p= 0.0001)

3.4 The regression equation of correlation of prednisolone dose and SLEDAI scores was proposed as the following:

Prednisolone dose (mg/week) = 10.509 SLEDAI +45.58 (p= 0.0001)

4. From pharmacodynamic study of NAC in mild SLE patients it could be concluded that

4.1 Administration of NAC may help to tapering prednisolone dosage.

4.2 Significant of its lower MDA effect in NAC group was observed. While no significant of GSH level was found. Further studies are needed to confirm these results.

4.3 The significant different of lowering SLEDAI score during 6 months in NAC group had been observed since the third of NAC administration.

References

1. Thorn research Inc., *N-acetylcysteine monograph.* Alternative Medicine Review, 2000. **5**: p. 467-471.
2. Kidd PM. , *Gluthathione:Systemic protectant against oxidative and free radical damage.* Alternative Medicine Review, 1997. **2**: p. 155-176.
3. Gregory SK. , *Clinical application of N-acetylcysteine.* Alternative Medicine Review, 1998. **2**: p. 114-127.
4. Holdiness MR. , *Clinical Pharmacokinetics of N-acetylcysteine.* Clin Pharmacokinet, 1991. **20**: p. 123-134.
5. Zafarullah M. , et al., *Molecular mechanism of N-acetylcysteine actions.* Cell Mol. Life Sci, 2003. **60**: p. 6-20.
6. Banaclocha MM. , *Therapeutic potential of N-acetylcysteine in age related mitochondria in age related neurodegenerative disease.* Medical hypothesis, 2001. **56**: p. 472-477.
7. Perl A., et al., *Mitochondrial hyperpolarization:a checkpoint of T-cell life, death and autoimmunity.* Trends in immunology, 2004. **25**: p. 360-7.
8. Evans MD. , et al., *Aberrant processing of oxidative DNA damage in systemic lupus erythematosus.* Biochemical and biophysical research communications, 2000. **273**: p. 894-8.
9. Suwannaroj S. , et al., *Antioxidants suppress mortality in the female NZB x NZWF1 mouse model of systemic lupus erythematosus (SLE).* Lupus, 2001. **10**: p. 258-265.
10. Gill JM. , et al., *Diagnosis of Systemic Lupus Erythematosus.* American Academy Family Physicians, 2003. **68**: p. 2179-2186.
11. Lawrence RC. , et al., *Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States.* Arthritis Rheum, 1998. **41**: p. 778-799.
12. McCarty DJ. , et al., *Incidence of systemic lupus erythematosus, Race and gender differences.* Arthritis Rheum, 1995. **38**: p. 1260-1270.

13. Hsii FP. *Systemic Lupus Erythematosus*. in 10th APAR Congress of Rheumatology. 2002. Bangkok, Thailand: Rheuma21st.com.

14. Sirikong M. , et al., *Association of HLA-DRB1*1502-DQB1*0501 haplotype with susceptibility to systemic lupus erythematosus in Thais.* Tissue antigen, 2002. **59**: p. 113-117.

15. Gray-McGuire C. , et al., *Genome scan of human systemic lupus erythematosus by regression modeling evidence linkage and epistasis at 4p16-15.2.* Am J Hum Gent, 2000. **67**: p. 1460-1469.

16. Perdriger A. , Werner-Leyval S. , and Rollot-Elamrani K., *The genetic basis for systemic lupus erythematosus.* Joint Bone Spine, 2003. **70**: p. 103-108.

17. Rivest C. , et al., *Association between clinical factors, socioeconomic status and organ damage in recent onset systemic lupus erythematosus.* J Rheumatol, 2000. **27**: p. 680-684.

18. Janwityanuchit S., et al., *Infection in systemic lupus erythematosus.* J Med Assoc Thai, 1993: p. 542-548.

19. Wongchinsri J., et al., *Infection in Thai patients with systemic lupus erythematosus:a review of hospitalized patients.* J Med Assoc Thai, 2002. **85 suppl 1**: p. S34-S39.

20. Kasitanon N., et al., *Causes of death and prognostic factors in Thai patients with systemic lupus.* Asian Pac J Allergy Immunol, 2002. **20**: p. 85-91.

21. D'Cruz D, Khamashta MA, and Hughes GR, *Systemic lupus erythematosus.* Lancet, 2007. **369**: p. 587-595.

22. Cervera R and F. J, *Therapeutic perspectives in systemic lupus erythematosus.* Current Rheumatology Reviews, 2005. **1**: p. 45-47.

23. Mok, C.C. and C.S. Lau, *Pathogenesis of systemic lupus erythematosus.* J Clin Pathol, 2003. **56**(7): p. 481-90.

24. Kelley, V.R. and R.P. Wuthrich, *Cytokines in the pathogenesis of systemic lupus erythematosus.* Semin Nephrol, 1999. **19**(1): p. 57-66.

25. Tenbrock, K., et al., *Altered signal transduction in SLE T cells.* Rheumatology (Oxford), 2007. **46**(10): p. 1525-30.

26. Taysi S., et al., *Serum oxidant/antioxidant status in patients with systemic lupus erythematosus.* Clin Chem Lab Med, 2002. **40**: p. 684-688.

27. North DS, Peterson RG, and Krenzelok EP, *Effect of activated charcoal administration on acetylcysteine serum level in humans.* American Journal of Hospital Pharmacy, 1981. **38**: p. 1022-1024.

28. Klein-Schwartz W and Odera GM, *Adsorption of oral antidotes for acetaminophen poisoning (methionine and N-acetylcysteine) by activated chacoal.* Clinical Toxicology, 1981. **18**: p. 283-290.

29. Jones A L., et al., *Pharmacokinetics of N-acetylcysteine are altered in patients with chronic liver disease.* Ailment Pharmacol Ther, 1997. **11**: p. 787-791.

30. De Caro, L., et al., *Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers.* Arzneimittelforschung, 1989. **39**(3): p. 382-6.

31. Bergstrom, A.L., L.B. Olsson, and J. Kutti, *Platelet survival and platelet production in systemic lupus erythematosus (SLE).* Scand J Rheumatol, 1980. **9**(4): p. 209-15.

32. De Bernardi di Valserra, M., et al., *Bioavailability of suckable tablets of oral N-acetylcysteine in man.* Eur J Clin Pharmacol, 1989. **37**(4): p. 419-21.

33. Borgstrom, L., B. Kagedal, and O. Paulsen, *Pharmacokinetics of N-acetylcysteine in man.* Eur J Clin Pharmacol, 1986. **31**(2): p. 217-22.

34. Pendyala L and Creavan PJ, *Pharmacokinetic and Pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent during a Phase I trial.* Cancer Epidemiology, Biomarker & Prevention, 1995. **4**: p. 245-251.

35. Aitio ML, *N-acetylcsyteine-pass-partout or much ado about nothing?* British journal of clinical pharmacology, 2006. **61**: p. 5-15.

36. Demedts M, et al., *High dose acetylcysteine in idiopathic pulmonary fibrosis.* N Engl J Med, 2005. **353**: p. 2229-2242.

37. Atkuri, K.R., et al., *N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency.* Curr Opin Pharmacol, 2007. **7**(4): p. 355-9.

38. De Rosa, S.C., et al., *N-acetylcysteine replenishes glutathione in HIV infection.* Eur J Clin Invest, 2000. **30**(10): p. 915-29.

39. Raju, P.A., et al., *Glutathione precursor and antioxidant activities of N-acetylcysteine and oxothiazolidine carboxylate compared in in vitro studies of HIV replication.* AIDS Res Hum Retroviruses, 1994. **10**(8): p. 961-7.

40. Roederer, M., et al., *N-acetylcysteine: a new approach to anti-HIV therapy.* AIDS Res Hum Retroviruses, 1992. **8**(2): p. 209-17.

41. Raju, P.A., L.A. Herzenberg, and M. Roederer, *Glutathione precursor and antioxidant activities of N-acetylcysteine and oxothiazolidine carboxylate compared in in vitro studies of HIV replication.* AIDS Res Hum Retroviruses, 1994. **10**(8): p. 961-7.

42. De Folora S., Bennicelli C., and Camoirano A., *In vivo effects of N-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or metagenic compound.* Carcinogenesis, 1985. **6**: p. 1735-1745.

43. De Flora, S., et al., *In vitro effects of N-acetylcysteine on the mutagenicity of direct-acting compounds and procarcinogens.* Carcinogenesis, 1984. **5**(4): p. 505-510.

44. D'Agostini, F., et al., *Inhibition by oral N-acetylcysteine of doxorubicin-induced clastogenicity and alopecia, and prevention of primary tumors and lung micrometastases in mice.* Int J Oncol, 1998. **13**(2): p. 217-24.

45. Doroshow, J.H., et al., *Prevention of doxorubicin cardiac toxicity in the mouse by N-acetylcysteine.* J Clin Invest, 1981. **68**(4): p. 1053-64.

46. Wanamarta, A.H., et al., *Effect of N-acetylcysteine on the antiproliferative action of X-rays or bleomycin in cultured human lung tumor cells.* J Cancer Res Clin Oncol, 1989. **115**(4): p. 340-4.

47. Zandwijk NV., et al., *EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer.* J Nalt Cancer Inst, 2000. **92**: p. 977-86.

48. Kobrinsky NL, Harfield D., and Horner H., *Treatment of advanced malignancies with high dose acetaminophen and aceylcysteine recue.* Cancer Invest, 1996. **14**: p. 202-210.

49. De Folora S, Grassi C, and Carati L, *Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-tern N-acetylcysteine treatment.* Eur Respir J, 1997. **10**: p. 1535-1541.

50. Gavish, D. and J.L. Breslow, *Lipoprotein(a) reduction by N-acetylcysteine.* Lancet, 1991. **337**(8735): p. 203-4.

51. Wiklund, O., et al., *N-acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein(a) levels.* Atherosclerosis, 1996. **119**(1): p. 99-106.

52. Bostom AG., Shemin D., and Yoburn D., *Lack of effect of oral N-acetylcysteine on the acute dialysis-related lowering of total plasma homocysteine in hemodialysis patients.* Atherosclerosis, 1996. **120**: p. 241-244.

53. Ceconi, C., et al., *The role of glutathione status in the protection against ischaemic and reperfusion damage: effects of N-acetyl cysteine.* J Mol Cell Cardiol, 1988. **20**(1): p. 5-13.

54. Sochman, J., et al., *Infarct Size Limitation: acute N-acetylcysteine defense (ISLAND trial): preliminary analysis and report after the first 30 patients.* Clin Cardiol, 1996. **19**(2): p. 94-100.

55. Rogers, D.F., et al., *Oral N-acetylcysteine speeds reversal of cigarette smoke-induced mucous cell hyperplasia in the rat.* Exp Lung Res, 1988. **14**(1): p. 19-35.

56. Moldeus, P., I.A. Cotgreave, and M. Berggren, *Lung protection by a thiol-containing antioxidant: N-acetylcysteine.* Respiration, 1986. **50 Suppl 1**: p. 31-42.

57. Eklund, A., et al., *Oral N-acetylcysteine reduces selected humoral markers of inflammatory cell activity in BAL fluid from healthy smokers: correlation to effects on cellular variables.* Eur Respir J, 1988. **1**(9): p. 832-8.

58. Selvan, V.A., et al., *Weight-based N-acetylcysteine dosing chart to minimise the risk of calculation errors in prescribing and preparing N-acetylcysteine infusions for adults presenting with paracetamol overdose in the emergency department.* Emerg Med J, 2007. **24**(7): p. 482-4.

59. Wilkes, J.M., L.E. Clark, and J.L. Herrera, *Acetaminophen overdose in pregnancy.* South Med J, 2005. **98**(11): p. 1118-22.

60. Kozer, E. and M. McGuigan, *Treatment strategies for early presenting acetaminophen overdose: a survey of medical directors of poison centers in North America and Europe.* Hum Exp Toxicol, 2002. **21**(3): p. 123-7.

61. Kozer, E. and G. Koren, *Management of paracetamol overdose: current controversies.* Drug Saf, 2001. **24**(7): p. 503-12.

62. Casey, P.B. and J.A. Tracey, *N-acetylcysteine (NAC)--a safe antidote in paracetamol poisoning?* Ir Med J, 1997. **90**(1): p. 38.

63. Patrick, L., *Toxic metals and antioxidants: Part II. The role of antioxidants in arsenic and cadmium toxicity.* Altern Med Rev, 2003. **8**(2): p. 106-28.

64. Hjortso, E., J.S. Fomsgaard, and N. Fogh-Andersen, *Does N-acetylcysteine increase the excretion of trace metals (calcium, magnesium, iron, zinc and copper) when given orally?* Eur J Clin Pharmacol, 1990. **39**(1): p. 29-31.

65. Ochsendorf, F.R. and U. Runne, *[The therapy of systemic lupus erythematosus].* Dtsch Med Wochenschr, 1997. **122**(27): p. 877.

66. Stanislaus, R., et al., *N-acetyl-L-cysteine ameliorates the inflammatory disease process in experimental autoimmune encephalomyelitis in Lewis rats.* J Autoimmune Dis, 2005. **2**(1): p. 4.

67. Walters MT., et al., *A double-blind cross-over study of oral N-acetylcysteine in Sjogren's syndrome.* Scand J Rheumatol Suppl, 1986. **61**: p. 253-258.

68. Fishbane S, Durham JH, and Marzo K, *N-acetylcysteine in the prevention of radiocontrast-induced nephropathy.* J Am Soc Nephrol, 2004. **15**: p. 251-260.

69. Bagshaw SM. and Ghali WA. *Acetylcysteine for prevention of contrast-induced nephropathy after intravascular angiogaraphy:Asystemic review and meta-analysis*. 2004 [cited 2005 3 March].

70. Baker, C.S., et al., *A rapid protocol for the prevention of contrast-induced renal dysfunction: the RAPPID study.* J Am Coll Cardiol, 2003. **41**(12): p. 2114-8.

71. Diaz-Sandoval, L.J., B.D. Kosowsky, and D.W. Losordo, *Acetylcysteine to prevent angiography-related renal tissue injury (the APART trial).* Am J Cardiol, 2002. **89**(3): p. 356-8.

72. Kay, J., et al., *Acetylcysteine for prevention of acute deterioration of renal function following elective coronary angiography and intervention: a randomized controlled trial.* Jama, 2003. **289**(5): p. 553-8.

73. Shyu, K.G., J.J. Cheng, and P. Kuan, *Acetylcysteine protects against acute renal damage in patients with abnormal renal function undergoing a coronary procedure.* J Am Coll Cardiol, 2002. **40**(8): p. 1383-8.

74. Tepel, M., et al., *Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine.* N Engl J Med, 2000. **343**(3): p. 180-4.

75. Allaqaband, S., et al., *Prospective randomized study of N-acetylcysteine, fenoldopam, and saline for prevention of radiocontrast-induced nephropathy.* Catheter Cardiovasc Interv, 2002. **57**(3): p. 279-83.

76. Boccalandro, F., et al., *Oral acetylcysteine does not protect renal function from moderate to high doses of intravenous radiographic contrast.* Catheter Cardiovasc Interv, 2003. **58**(3): p. 336-41.

77. Briguori, C., et al., *Acetylcysteine and contrast agent-associated nephrotoxicity.* J Am Coll Cardiol, 2002. **40**(2): p. 298-303.

78. Durham, J.D., et al., *A randomized controlled trial of N-acetylcysteine to prevent contrast nephropathy in cardiac angiography.* Kidney Int, 2002. **62**(6): p. 2202-7.

79. Goldenberg, I., et al., *Oral acetylcysteine as an adjunct to saline hydration for the prevention of contrast-induced nephropathy following coronary angiography. A randomized controlled trial and review of the current literature.* Eur Heart J, 2004. **25**(3): p. 212-8.

80. Loutrianakis E, et al., *Randomized comparison of fenaldopam and n-acetylcysteine to saline in the prevention of radiocontrast nephropathy [Abstract].* J Am Coll Cardiol, 2003. **41**: p. 327A.

81. Oldemeyer, J.B., et al., *Acetylcysteine in the prevention of contrast-induced nephropathy after coronary angiography.* Am Heart J, 2003. **146**(6): p. E23.

82. Vallero, A., et al., *[Contrast nephropathy in cardiac procedures: no advantages with prophylactic use of N-acetylcysteine (NAC)].* G Ital Nefrol, 2002. **19**(5): p. 529-33.

83. Droge, W. and R. Breitkreutz, *Glutathione and immune function.* Proc Nutr Soc, 2000. **59**(4): p. 595-600.

84. Pastore A., et al., *Analysis of glutathione:implication in redox and detoxification.* Clinica Chimica Acta, 2003. **333**: p. 19-39.

85. Lang CA , et al., *Low blood Glutathione level in healthy aging adults.* J Lab Clin Med, 1992. **120**: p. 720-725.

86. Chavan S, et al., *Reduced Glutathione: Importance of specimen collection.* Indain Journal of Clinical Biochemistry, 2005. **20**: p. 150-152.

87. Rossi R , et al., *Blood glutathione disulfide: In vivo factor or in vitro artifact.* Clin Chem, 2002. **48**: p. 742-753.

88. Lykkesfeldt J, *Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking.* Clinica Chemica Acta, 2007. **380**: p. 50-58.

89. Del Rio D, Stewart AJ, and Pellegrini N, *A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress.* Nutrition, metabolism and cardiovascular disease, 2005. **15**: p. 316-328.

90. Rasheed MH, Beevi SS, and Geetha A, *Enhanced lipid peroxidation and nitric oxide product with deranged antioxidant status in patients with head and neck squamous cell carcinoma.* Oral Oncology, 2007. **43**: p. 333-338.

91. Manoharan S, et al., *Lipid peroxidation and antioxidants status in patients with oral squamous cell carcinoma.* Indian J Med Res, 2005. **122**: p. 529-534.

92. Yoneyama Y, et al., *Relationship between plasma malondialdehyde levels and adenosine deaminase activities in preeclampsia.* Clin Chim Acta, 2002. **322**: p. 169-173.

93. Slatter DA, Bolton CH, and Bailey AJ, *The importance of lipid-derived malondialdehyde in diabetes mellitus [Review].* Diabetologia, 2000. **43**: p. 550-557.

94. Polidori MC, et al., *Plasma lipophillic antioxidants and malondialdehyde in congestive heart failure patients:relationship to disease severity.* Free Radic Bio Med 2002. **32**: p. 148-152.

95. Delibas N, Ozcankaya R, and Altuntas I, *Clinical importance of erythrocyte malondialdehyde level as a marker for cognitive deterioration in patients with dementia of alzhimer type: a repeated study in 5 year interval.* Clin Biochem, 2002. **35**: p. 137-141.

96. Polat, G., et al., *Levels of malondialdehyde, glutathione and ascorbic acid in idiopathic thrombocytopaenic purpura.* East Afr Med J, 2002. **79**(8): p. 446-9.

97. Stefanescu, M., et al., *Pycnogenol efficacy in the treatment of systemic lupus erythematosus patients.* Phytother Res, 2001. **15**(8): p. 698-704.

98. Donne DI , et al., *Biomarkers of oxidative damage in human disease.* Clin Chem, 2006. **52**: p. 601-623.

99. Jekel JF. , Elmore JG. , and Kate DL., *Sample size, Randomization, and Probability Theory*, in Epidimiology biostatistic and preventive medicine, James F. Jekel, Joann G. Elmore, and D.L. Kate, Editors. 1996, W.B. Sauders company: NewYork, USA. p. 160-169.

100. Yee, C.S., et al., *EULAR randomised controlled trial of pulse cyclophosphamide and methylprednisolone versus continuous cyclophosphamide and prednisolone followed by azathioprine and prednisolone in lupus nephritis.* Ann Rheum Dis, 2004. **63**(5): p. 525-9.

101. Kumar A. , *Indian guidelines on the management of SLE.* J Indian Rheumatol Assoc, 2002. **10**: p. 80-96.

102. Bae SC. , Kim SJ. , and Sung MK. , *Impaired antioxidant status and decreased dietary intake of antioxidants in patients with systemic lupus erythematosus.* Rheumatol Int, 2002. **22**: p. 238-43.

103. Institueforalgorithmicmedicine. *Algorithm for systemic lupus erythematosus (SLE) disease activity index*. 2007 [cited 2007 2 June]; Available from: http://www,medal.org/visitor/login.aspx.

104. D'Cruz D, Khamashta MA, and Hughes G, *Systemic lupus erythematosus.* Lancet, 2007. **17**: p. 587-596.

105. Dooley, M.A. and E.M. Ginzler, *Newer therapeutic approaches for systemic lupus erythematosus: immunosuppressive agents.* Rheum Dis Clin North Am, 2006. **32**(1): p. 91-102, ix.

106. Tahir, H. and D.A. Isenberg, *Novel therapies in lupus nephritis.* Lupus, 2005. **14**(1): p. 77-82.

107. Parker, B.J. and I.N. Bruce, *High dose methylprednisolone therapy for the treatment of severe systemic lupus erythematosus.* Lupus, 2007. **16**(6): p. 387-93.

108. Buhaescu, I., A. Covic, and G. Deray, *Treatment of proliferative lupus nephritis--a critical approach.* Semin Arthritis Rheum, 2007. **36**(4): p. 224-37.

109. Barile-Fabris, L., et al., *Controlled clinical trial of IV cyclophosphamide versus IV methylprednisolone in severe neurological manifestations in systemic lupus erythematosus.* Ann Rheum Dis, 2005. **64**(4): p. 620-5.

110. Beimler, J.H. and K. Andrassy, *Cyclophosphamide treatment in systemic necrotizing vasculitis and lupus nephritis. How long? How much?* Pediatr Nephrol, 2004. **19**(9): p. 949-55.

111. Costedoat-Chalumeau, N., et al., *Hydroxychloroquine in systemic lupus erythematosus.* Lancet, 2007. **369**(9569): p. 1257-8.

112. Alarcon, G.S., et al., *Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (LUMINA L).* Ann Rheum Dis, 2007. **66**(9): p. 1168-72.

113. Clowse, M.E., et al., *Hydroxychloroquine in lupus pregnancy.* Arthritis Rheum, 2006. **54**(11): p. 3640-7.

114. Costedoat-Chalumeau, N., et al., *Low blood concentration of hydroxychloroquine is a marker for and predictor of disease exacerbations in patients with systemic lupus erythematosus.* Arthritis Rheum, 2006. **54**(10): p. 3284-90.

115. Calvo-Alen, J., et al., *Systemic lupus erythematosus in a multiethnic US cohort (LUMINA): XXIV. Cytotoxic treatment is an additional risk factor for the development of symptomatic osteonecrosis in lupus patients: results of a nested matched case-control study.* Ann Rheum Dis, 2006. **65**(6): p. 785-90.

116. Petri, M., *Immunosuppressive drug use in pregnancy.* Autoimmunity, 2003. **36**(1):p. 51-6.

117. Rahman, P., et al., *The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs.* J Rheumatol, 1999. **26**(2): p. 325-30.

118. Clowse, M.E., *Lupus activity in pregnancy.* Rheum Dis Clin North Am, 2007. **33**(2): p. 237-52, v.

119. Ranchin, B. and S. Fargue, *Review: New treatment strategies for proliferative lupus nephritis: keep children in mind!* Lupus, 2007. **16**(8): p. 684-91.

120. Spertini, F., *[New concepts for the therapy of systemic lupus erythematosus].* Rev Med Suisse, 2007. **3**(94): p. 98-102.

121. Wozniacka, A., et al., *The dynamism of cutaneous lupus erythematosus: mild discoid lupus erythematosus evolving into SLE with SCLE and treatment-resistant lupus panniculitis.* Clin Rheumatol, 2007. **26**(7): p. 1176-9.

122. Bobrowska-Snarska, D., et al., *[Severe neurological and obstetrical complications in a patient with antiphospholipid syndrome].* Pol Arch Med Wewn, 2006. **115**(5): p. 457-62.

123. Wozniacka, A. and D.P. McCauliffe, *Optimal use of antimalarials in treating cutaneous lupus erythematosus.* Am J Clin Dermatol, 2005. **6**(1): p. 1-11.

124. Borba, E.F., J.F. Carvalho, and E. Bonfa, *Mechanisms of dyslipoproteinemias in systemic lupus erythematosus.* Clin Dev Immunol, 2006. **13**(2-4): p. 203-8.

125. El-Sehemy, M.S., et al., *Comparative clinical prospective therapeutic study between cyclophosphamide, cyclosporine and azathioprine in the treatment of lupus nephritis.* Egypt J Immunol, 2006. **13**(1): p. 39-52.

126. Heath, M. and G.J. Raugi, *Evidence-based evaluation of immunomodulatory therapy for the cutaneous manifestations of lupus.* Adv Dermatol, 2004. **20**: p. 257-91.

127. Contreras, G., et al., *Lupus nephritis: a clinical review for practicing nephrologists.* Clin Nephrol, 2002. **57**(2): p. 95-107.

128. Houssiau, F.A., *Management of refractory systemic rheumatic diseases.* Acta Clin Belg, 2003. **58**(5): p. 314-7.

129. Sultan, S.M., Y. Ioannou, and D.A. Isenberg, *Is there an association of malignancy with systemic lupus erythematosus? An analysis of 276 patients under long-term review.* Rheumatology (Oxford), 2000. **39**(10): p. 1147-52.

130. Yeap, S.S., et al., *Comparison of treatment regimes for lupus nephritis.* Med J Malaysia, 2002. **57**(3): p. 311-8.

131. Abu-Shakra, M. and Y. Shoenfeld, *Azathioprine therapy for patients with systemic lupus erythematosus.* Lupus, 2001. **10**(3): p. 152-3.

132. McLigeyo, S.O., *Treatment options in lupus nephritis.* East Afr Med J, 1998. **75**(10): p. 609-13.

133. Gladman D. , et al., *The development and initial validation of the systemic lupus international collaborating clinic/American college of rheumatology damage index for systemic lupus erythematosus.* Arthritis&Rheumatism, 1996. **39**: p. 364-369.

134. Benseler, S.M. and E.D. Silverman, *Review: Neuropsychiatric involvement in pediatric systemic lupus erythematosus.* Lupus, 2007. **16**(8): p. 564-71.

135. Khamashta, M.A., *Systemic lupus erythematosus and pregnancy.* Best Pract Res Clin Rheumatol, 2006. **20**(4): p. 685-94.

136. Mukherjee, T.K., G.W. Fuller, and G.W. Friars, *Appraisal of collection techniques and storage temperatures on Turkey plasma chlolinesterase level and blood glutathione concentration.* Can J Comp Med., 1969. **33**: p. 233-234.

137. Czock D, et al., *Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids.* Clinical Pharmacokinetics, 2005. **44**: p. 61-98.

138. Frostegard J. , et al., *Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestation.* Arthritis Rheum, 2005. **52**: p. 192-200.

139. Cooke MS., Evans MD., and Dizdaroglu M., *Oxidative DNA damage:mechanism, mutation, and disease.* The FRSEB Jornal, 2003. **17**: p. 1195-1241.

140. Kurien BT. and Scofield RH., *Free radical mediated peroxidative damage in systemic lupus erythematosus.* Life Science, 2003. **73**: p. 1655-66.

141. Hultberg M and Hultberg B, *The effect of different antioxidants on glutathione turnover in human cell line and their interaction with hydrogen peroxide.* Chemico Biological Interaction, 2006. **163**: p. 192-198.

142. Block, K.I., et al., *Impact of antioxidant supplementation on chemotherapeutic efficacy: a systematic review of the evidence from randomized controlled trials.* Cancer Treat Rev, 2007. **33**(5): p. 407-18.

143. Atmaca, G., *Antioxidant effects of sulfur-containing amino acids.* Yonsei Med J, 2004. **45**(5): p. 776-88.

144. Karlson, E.W., et al., *Effect of glutathione S-transferase polymorphisms and proximity to hazardous waste sites on time to systemic lupus erythematosus diagnosis: results from the Roxbury lupus project.* Arthritis Rheum, 2007. **56**(1): p. 244-54.

145. Zhong, S., et al., *Relationship of glutathione S-transferase genotypes with side-effects of pulsed cyclophosphamide therapy in patients with systemic lupus erythematosus.* Br J Clin Pharmacol, 2006. **62**(4): p. 457-72.

146. Kang I. and Park SH. , *Infectious complications in SLE after immunosuppressive therapies.* Curr Opin Rheumatol, 2003. **15**: p. 528-534.

Appendices

Appendix A

Table 1 Comparison of proportion of SLE patients who received and no receive prednisolone containing regimens relate to outcomes

1a. Mild SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	Active	Death	
Received	50 (79.36)	7 (11.11)	6 (9.52)	0 (0.00)	63
No	42 (93.33)	3 (6.67)	0 (0.00)	0 (0.00)	45
Total	**92**	**10**	**6**	**0**	**108**

1b. Moderate SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	Active	Death	
Received	66 (85.71)	7 (9.09)	4 (5.19)	0 (0.00)	77
No	61 (85.92)	6 (8.45)	4 (5.63)	0 (0.00)	71
Total	**127**	**13**	**8**	**0**	**148**

1c. Severe SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	Active	Death	
Received	12 (85.71)	2 (14.29)	0 (0.00)	0 (0.00)	14
No	9 (75.00)	1 (8.33)	1 (8.33)	1 (8.33)	12
Total	**21**	**3**	**1**	**1**	**26**

Table 2 Comparison of proportion of SLE patients who received chloroquine containing regimens relate to outcomes

2a. Mild SLE

Group	Out comes N (%)				Total
	Remission or inactive	Improved	active	Death	
Received	11 (73.33)	3 (20.00)	1 (6.67)	0 (0.00)	15
No	81 (87.10)	7 (7.50)	5 (5.38)	0 (0.00)	93
Total	**92**	**10**	**6**	**0**	**108**

2b. Moderate SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	Active	death	
Received	7 (46.67)	6 (40.00)	2 (13.33)	0 (0.00)	15
No	120 (9.02)	7 (5.26)	6 (4.51)	0 (0.00)	133
Total	**127**	**13**	**8**	**0**	**148**

2c. Severe SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	death	
Received	1 (33.33)	2 (66.67)	0 (0.00)	0 (0.00)	3
No	20 (86.96)	1 (4.36)	1 (4.35)	1 (4.35)	23
Total	**21**	**3**	**1**	**1**	**26**

Table 3 Comparison of proportion of SLE patients who received azathioprine containing regimens relate to outcomes

3a. Mild SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	Death	
Received	15 (93.75)	0 (0.00)	1 (6.25)	0 (0.00)	16
No	77 (83.69)	10 (10.87)	5 (5.43)	0 (0.00)	92
Total	**92**	**10**	**6**	**0**	**108**

3b. Moderate SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	death	
Received	23 (92.00)	1 (4.00)	2 (8.00)	0 (0.00)	25
No	104 (84.55)	12(7.76)	6 (4.88)	1 (0.00)	123
Total	**127**	**13**	**8**	**1**	**148**

3c. Severe SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	death	
Received	1 (50.00)	1 (50.00)	0 (0.00)	0 (0.00)	2
No	20 (83.33)	2 (4.55)	1 (4.17)	1 (4.17)	24
Total	**21**	**3**	**1**	**1**	**26**

Table 4 Comparison of proportion of SLE patients who received hydroxychloroquine containing regimens relate to outcomes

4a. Mild SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	Death	
Received	16 (94.12)	0 (0.00)	1 (5.88)	0 (0.00)	17
No	76 (83.52)	10 (10.99)	5 (5.49)	0 (0.00)	91
Total	**92**	**10**	**6**	**0**	**108**

4b. Moderate SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	death	
Received	26 (92.85)	2 (7.14)	0 (0.00)	0 (0.00)	28
No	101 (84.17)	9 (7.50)	8 (6.67)	0 (0.00)	120
Total	**127**	**11**	**8**	**0**	**148**

4c. Severe SLE

Group	Out comes, N (%)				Total
	Remission or inactive	Improved	active	death	
Received	4 (100.00)	0 (0.00)	0 (0.00)	0 (0.0)	4
No	17 (77.27)	3 (13.64)	1 (4.45)	1 (4.54)	22
Total	**21**	**3**	**1**	**0**	**26**

Appendix B

Table 5 Plasma Malondialdehyde (MDA) Concentration (μM) in Systemic Lupus Erythematosus (SLE) patients who did not receive NAC

Subject No	Base line	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
1	0.265		0.520	0.279			0.269	0.317
2	0.981	0.388	1.129	0.171	0.255			0.365
3	0.347			0.967		0.166		0.299
4	0.235			0.217			0.272	0.288
5	0.619	0.753		0.422	0.445	0.259	0.110	0.399
6	0.522		0.195	0.296	0.344	0.725	0.245	0.550
7	0.265		0.192	0.369	0.171	0.334	0.511	0.296
8	0.331			0.311				0.479
9	0.592	0.204		0.318		0.287	0.159	0.420
10	0.797		0.416	0.252	0.004			1.181
11	0.235	0.248	0.030	0.328	0.367	0.163		0.346
12	0.361				0.322	0.333		1.149
13	0.490	0.922	0.959	0.195	0.212	0.628		0.380
14	0.550			0.168			0.364	0.317
15	0.341		2.027		0.153	0.547		0.217
16	0.464	0.469	0.310	0.828	0.381		0.518	0.590
17	0.789							0.278
18	0.216						0.630	0.438
19	0.141			0.282				0.927
20	0.239				0.503			0.197
Mean	0.439	0.497	0.590	0.366	0.286	0.362	0.342	0.510
SD	0.227	0.285	0.633	0.238	0.138	0.203	0.177	0.286
SEM	0.057	0.116	0.211	0.064	0.037	0.054	0.047	0.076

Table 6 Plasma Malondialdehyde (MDA) Concentration (μM) in Systemic Lupus Erythematosus (SLE) patients who received NAC

Subject No	Base line	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
1	0.583		0.244	0.611		0.303		0.214
2	0.980		1.444	0.670		0.799		0.239
3	1.007		0.255	0.053	0.279			0.844
4	0.898		0.207	0.348	0.172	0.308		0.762
5	0.631	0.155	0.240	0.259		0.303		0.308
6	0.697	0.210	0.244	0.319	1.018	0.280	0.649	0.256
7	0.621		0.191	0.618			0.445	0.221
8	0.217		0.374	0.376			0.231	0.331
9	0.344			0.257	1.238		0.390	0.420
10	0.314	0.268	0.321	0.269	0.516		0.347	0.290
11	0.292		0.223		0.351		0.295	0.268
12	0.189					0.356		0.329
13	0.346		0.560			0.387		0.318
14	0.868			0.296	0.454		0.589	0.135
15	0.327	0.242			0.221			0.233
16	0.651		0.311	0.303	0.339		0.358	0.367
17	0.280			0.265		0.696		0.358
18	0.450		0.256	0.214				0.530
19	0.189					0.327		0.419
20	0.246				0.311			0.233
Mean	0.507	0.219	0.375	0.357	0.510	0.429	0.413	0.354
SD	0.274	0.049	0.336	0.172	0.355	0.191	0.143	0.178
SEM	0.068	0.022	0.101	0.048	0.098	0.053	0.040	0.049

Table 7 Plasma Glutathione (GSH) concentration (μM)in Systemic Lupus Erythematosus (SLE) patients who did not receive NAC

Subject No	Base line	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
1	471.90		525.80	478.74			319.22	483.72
2	621.78	350.20	387.40	332.99	252.06			478.32
3	484.59			269.13		625.12		545.53
4	409.94			277.53			200.46	473.67
5	570.20	304.19		1068.57	491.65	831.12	637.11	480.44
6	452.71		518.34	215.67	214.73	678.11	413.48	418.57
7	356.93		690.13	218.24	506.03	319.24	475.43	3625.45
8	512.0			570.95				269.19
9	340.29	443.89		634.72		642.55	626.06	786.66
10	1964.87		386.35	606.44	744.18			1109.6
11	898.59	629.007	746.07	455.12	461.9	681.82		342.29
12	784.23				894.56	1359.66		1348.4
13	786.90	243.10	469.70	829.44	508.50	161.72		219.76
14	533.46			406.63			394.94	879.97
15	269.99		634.07		526.08	641.61		839.61
16	82.08	423.03	379.17	263.73	468.76		1712.57	1070.31
17	499.51							813.73
18	350.56						268.84	1063.76
19	434.62				210.89			766.64
20	588.67				340.51			761.83
Mean	570.69	398.90	526.34	473.42	468.32	660.11	560.90	838.87
SD	377.55	135.03	137.18	250.28	204.14	332.77	456.43	738.64
SEM	94.39	55.13	45.73	66.89	54.56	88.94	121.99	197.41

Table 8 Plasma Glutathione (GSH) concentration (μM) in Systemic Lupus Erythematosus (SLE) patients who received NAC

Subject No	Base line	2 weeks	1 month	2 months	3 months	4 months	5 months	6 months
1	692.78		366.95	384.43		680.48		521.39
2	409.31		364.12	328.90		784.16		948.46
3	516.73		1124.9	3040.5	1066.11			580.3
4	562.62		917.12	827.76	801.79	733.67		247.45
5	313.58	475.67	390.03	276.59		469.46		862.26
6	717.06	603.03	593.22	517.88	657.07	659.74	334.17	1234.3
7	409.93		753.14	570.67			418.41	893.39
8	491.12		488.03	391.31			469.93	503.7
9	498.33			432.91	274.67		314.40	370.27
10	1970.30	1367.92	745.04	396.486	478.49		442.69	325.33
11	509.87		611.12		572.82		473.98	2918.39
12	766.04					450.25		238.63
13	473.83		776.26			559.73		338.32
14	700.98			236.79	407.11		624.26	460.12
15	219.28	504.84			234.58			318.74
16	230.59		547.98	432.67	285.48		1095.42	517.81
17	2018.45			2251.00		226.152		613.46
18	438.72		584.49	675.41				353.28
19	1059.60					400.95		794.52
20	470.60				5282.15			1005.85
Mean	673.49	737.87	635.57	775.99	530.90	570.46	521.66	702.30
SD	491.31	423.56	224.52	824.87	1524.84	180.71	250.62	592.97
SEM	122.83	172.92	74.84	220.46	407.53	48.30	66.98	158.48

Table 9 Statistical analysis of effect of NAC on SLEDAI

Tests of Between-Subjects Effects

Dependent Variable: SLEDAI1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	600.357	1	600.357	43.730	.096	.978	43.730	.396
	Error	13.729	1	13.729(b)					
TIME	Hypothesis	66.043	6	11.007	98.362	.000	.990	590.170	1.000
	Error	.671	6	.112(c)					
GROUP2	Hypothesis	13.729	1	13.729	122.681	.000	.953	122.681	1.000
	Error	.671	6	.112(c)					
TIME *	Hypothesis	.671	6	.112	.066	.999	.001	.396	.066
GROUP2	Error	451.200	266	1.696(d)					

a Computed using alpha = .05

b MS(GROUP2)

c MS(TIME * GROUP2)

d MS(Error)

Table 10 Statistical analysis of effect of NAC on prednisolone dose.

Tests of Between-Subjects Effects

Dependent Variable: PRED1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	Hypothesis	13073077.565	1	13073077.565	47579.973	.003	1.000	47579.973	1.000
	Error	274.760	1	274.760(b)					
TIME	Hypothesis	28291970.514	7	4041710.073	9717.668	.000	1.000	68023.677	1.000
	Error	2911.395	7	415.914(c)					
GROUP2	Hypothesis	274.760	1	274.760	.661	.443	.086	.661	.109
	Error	2911.395	7	415.914(c)					
TIME * GROUP2	Hypothesis	2911.395	7	415.914	.052	1.000	.001	.365	.063
	Error	2357938.576	296	7966.009(d)					

a Computed using alpha = .05 b MS(GROUP2) c MS(TIME * GROUP2) d MS(Error)

BIOGRAPHY

Ms. Karunrat Tewthanom was born in 14 March 1973 at Lumpang province, Thailand. She graduated a Bachelor of Pharmacy (B. Pharm) with second honors from Silpakorn University in 1995. After that, she received a Master of Science in Pharmacy (Clinical Pharmacy) at Mahidol University in 1998. She have been enrolled in the doctor of philosophy in Pharmaceutical care (Ph.D. in Pharmaceutical care) at Faculty of Pharmaceutical Sciences, Chulalongkorn University, since 2002. Currently, she is an academic staff of the department of pharmacy, Faculty of Pharmacy, Silpakorn University, Snamchandra Palace campus, Nakhon Pathom, Thailand.